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"Congestion profiles in heart failure"

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Profils congestifs en insuffisance cardiaque.

Introduction

L'insuffisance cardiaque (IC) est un syndrome hétérogène complexe associé à une morbidité et une mortalité élevées. En France, on dénombre plus de 1,3 million de cas prévalents d'IC, soit environ 2,6 % de la population adulte. En 2022, près de 34 % des patients hospitalisés pour une IC sont décédés dans l'année qui a suivi leur hospitalisation. L'IC est le résultat de facteurs de risque tels que le diabète, l'obésité, le tabagisme, l'infarctus du myocarde, qui entraînent des anomalies structurelles et fonctionnelles du cœur.

La congestion est l'un des principaux facteurs de symptômes et d'hospitalisation dans l'IC. Elle est définie comme une augmentation des pressions de remplissage centrales qui résultent et contribuent à la surcharge volumique résultant de la rétention hydrosodée. Les signes et symptômes de la congestion sont la difficulté à respirer, le gonflement des chevilles, l'élévation de la pression veineuse jugulaire, les râles, l'œdème périphérique et l'élévation des peptides natriurétiques.

Physiopathologie de la congestion dans l'insuffisance cardiaque

Les mécanismes sous-jacents associés à la congestion sont complexes : l'interaction de plusieurs facteurs tels que les fonctions cardiaques et rénales, l'intégrité des membranes endothéliales, le rôle des compartiments interstitiels et extravasculaires jouent un rôle important dans la congestion. Les lésions cardiaques et le dysfonctionnement du myocarde entraînent une stase veineuse et capillaire qui perturbe les forces de Starling et l'hémodynamique microcirculatoire. Les modifications des forces hydrostatiques et oncotiques entraînent un déplacement de liquide de l'espace intravasculaire vers l'espace interstitiel. En outre, l'insuffisance de perfusion des organes, y compris les reins, active le système sympathique rénal, ce qui aggrave encore les symptômes congestifs. La réduction du volume circulant effectif et la diminution de la circulation dans l'artère rénale déclenchent une réaction compensatoire de rétention d'eau et de sel pour augmenter le volume plasmatique.

L'augmentation progressive et soutenue du liquide dans le compartiment vasculaire entraîne des fuites transcapillaires, le déplaçant des compartiments vasculaires vers les espaces extravasculaires, ce qui entraîne un œdème tissulaire. L'augmentation de la pression du cœur gauche entraîne une augmentation de la pression dans les veines et les capillaires pulmonaires. L'augmentation de la pression capillaire dans les poumons provoque une accumulation de liquide interstitiel périalvéolaire. Les signes et symptômes de la congestion pulmonaire comprennent une dyspnée, des râles, des crépitants et des lignes B à l'échographie pulmonaire. L'augmentation de la pression du cœur droit est étroitement liée à la congestion systémique, entraînant une élévation de la pression veineuse jugulaire et une dilatation de la veine cave inférieure (à l'échographie).

Étant donné l'hétérogénéité inhérente à la congestion au niveau des différents organes et la variabilité d'un patient à l'autre, il serait important de comprendre les différences dans les schémas de distribution des fluides dans les compartiments vasculaires et extravasculaires, ainsi que les différences dans l'accumulation des fluides aux niveaux systémiques et pulmonaires, afin d'élaborer de meilleures approches thérapeutiques.

Marqueurs, évaluation et classification de la congestion

Une évaluation intégrée de la congestion est nécessaire pour une prise en charge appropriée et la stratification du risque. Les outils d'évaluation de la congestion les plus courants sont les suivants:

Examen clinique - les marqueurs de la congestion à l'examen physique sont la présence de râles, d'œdèmes périphériques, d'orthopnée et d'une élévation de la pression veineuse jugulaire.

Des scores cliniques de congestion combinés - basés sur les marqueurs de l'examen physique ont été utilisés pour évaluer la gravité de la congestion. Ils offrent une évaluation plus précise de la gravité de la congestion mais sont associées de façon modérée au risque d’évènements.

Echocardiographie - c'est une condition préalable au diagnostic de l'IC. L'échocardiographie fournit des estimations de la pression de remplissage, de la taille des cavités cardiaques et de la fonction cardiaque. Les paramètres échocardiographiques tels que LAVi et E/e' se sont révélés être des marqueurs pronostiques indépendants dans l’IC. Cependant, la précision diagnostique de paramètres échocardiographiques individuels

pour diagnostiquer l'IC est limitée, en particulier dans l'HFpEF. L'algorithme de l'ASE/EACVI 2016 estime les pressions de remplissage du ventricule gauche en utilisant quatre paramètres échocardiographiques (E/A, E/e', TRV et LAVi), mais souffre de plusieurs limitations.

Échographie pulmonaire - il s'agit d'une méthode fiable et semi-quantitative pour estimer la congestion pulmonaire. Plusieurs études antérieures ont montré qu'un nombre élevé de lignes B est associé à un risque accru de mortalité ou d'hospitalisation pour IC. Des études récentes ont montré que le nombre de lignes B avait une corrélation modérée avec la pression auriculaire droite (r = 0,32), la pression diastolique de l'artère pulmonaire (r = 0,34), la pression moyenne de l'artère pulmonaire (r = 0,43) et la résistance vasculaire pulmonaire (r = 0,51) mesurées par cathétérisme cardiaque droit. Cependant, les lignes B ne sont pas spécifiques de l'IC et sont observées dans d'autres causes de désaération pulmonaire partielle telles que la fibrose pulmonaire, ainsi que le syndrome de détresse respiratoire aigüe et la pneumonie interstitielle. En outre, l'absence d'œdème pulmonaire n'exclut pas l'IC.

Taux de peptides natriurétiques - un taux élevé de peptides natriurétiques est l'un des critères de diagnostic de l'IC. Des valeurs de BNP supérieures à 35 pg/ml et de NTproBNP supérieures à 125 pg/ml indiquent une forte probabilité d'IC dans un contexte ambulatoire.

La classification de Forrester est utilisée pour classer les patients souffrant d'IC aiguë sur la base de l'évaluation de la congestion et de la perfusion au chevet du patient. Les patients sont classés comme humides ou secs en fonction de la présence d'une congestion et comme chauds ou froids en fonction de la présence ou de l'absence d'une hypoperfusion. Cependant, la classification de Forrester ne fait pas de distinction entre la congestion pulmonaire ou systémique et ne prend pas en compte d'autres facteurs de risque tels que les niveaux de peptides natriurétiques. Une classification des patients atteints d'IC en fonction des profils de congestion pourrait être utile pour stratifier le risque et personnaliser l'approche thérapeutique.

Malgré plusieurs outils d'évaluation de la congestion, il n'existe pas de méthode validée de segmentation de la congestion permettant d'identifier les profils de congestion. Cette lacune est probablement due au fait que

l'interaction complexe de multiples variables de congestion n'est pas toujours discernable par l'observation conventionnelle ou les approches analytiques conventionnelles. En outre, jusqu'à récemment, les statistiques médicales manquaient d'approches agnostiques/libres de tout a priori.

Bien que la congestion soit la principale cause d'hospitalisation dans l'IC, les marqueurs de congestion existants présentent une association modérée avec le pronostic des patients. Il est important de noter que les marqueurs de congestion disponibles guident rarement les décisions thérapeutiques, très probablement parce que notre compréhension de la congestion reste trop superficielle. Le traitement de l'IC guidée par les peptides natriurétiques n'a pas amélioré le prognose des patients dans les essais cliniques randomisés. **Il n'existe actuellement aucune méthode précise ou validée qui permette une segmentation précise de la congestion, nécessaire pour classer ou identifier les profils de congestion.** Cette lacune est probablement due au fait que l'interaction complexe de multiples variables de congestion n'est pas toujours discernable par l'observation ou les approches analytiques conventionnelles. Récemment, un cadre conceptuel a été proposé pour classer les patients en fonction du phénotype prédominant de la congestion, à savoir la congestion pulmonaire et systémique d'une part et tissulaire et intravasculaire d'autre part. Toutefois, ces concepts reposent sur l'opinion d'experts plutôt que sur un processus analytique fondé sur des données.

Rôle de l'intelligence artificielle dans l'identification des phénotypes de l'insuffisance cardiaque et des approches holistiques

Étant donné la complexité de la physiopathologie sous-jacente de la congestion, les modèles issus de l'IA pourraient fournir une meilleure vision des relations complexes entre les divers facteurs contributifs. Ils peuvent analyser un vaste ensemble de données afin d'identifier une population de patients plus homogène au sein de populations hétérogènes. Des modèles d'apprentissage automatique non supervisés tels que les K-means, le clustering ascendant hiérarchique, l'analyse des classes latentes et le modèle des classes latentes ont été utilisés pour identifier les profils d'IC ayant un profil clinique et un pronostic distincts. Des modèles

d'apprentissage automatique supervisés ont été utilisés pour développer des modèles de prédiction. Les modèles d'apprentissage automatique non supervisés les plus couramment utilisés pour identifier les phénotypes d'IC sont les K-means, le clustering ascendant hiérarchique, l'analyse de classe latente et le modèle des classes latentes. Le choix du modèle dépend de la taille et du type de données : Les K-means et le clustering hiérarchique conviennent mieux aux variables continues, tandis que le modèle d'analyse des classes latentes requiert des données sous forme catégorielle. Le modèle des classes latentes peut analyser des données hétérogènes composées à la fois de variables catégorielles et continues.

Les modèles basés sur l'apprentissage automatique ont été explorés pour toute une série d'applications en médecine, telles que le diagnostic, la prédiction de la réponse au traitement, la prédiction des effets indésirables et le phénotypage. Les modèles pilotés par l'IA ont la capacité d'analyser simultanément un large éventail de données dont des données biologiques. Cela pourrait permettre d'identifier des voies mécanistiques impliquées dans la progression de la maladie et la réponse thérapeutique. Toutefois, les difficultés inhérentes à l'élaboration et la présentation des algorithmes ainsi que la difficulté à valider dans des populations externes constituent un obstacle majeur au développement et à l'intégration de ces modèles dans la pratique clinique.

Contexte biologique de la congestion

Le rôle du système rénine-angiotensine-aldostérone (RAAS) a été largement étudié dans le contexte de l'IC. Le RAAS joue un rôle régulateur important dans le maintien de l'équilibre hydrosodée et la sécrétion d'aldostérone. Les médicaments qui inhibent le système RAAS, tels que les inhibiteurs de l'IEC, les bloqueurs des récepteurs de l'angiotensine et les antagonistes des minéralocorticoïdes, constituent la pierre angulaire du traitement de l'HFrEF. Ces médicaments réduisent le remodelage cardiaque et améliorent le pronostic de l'IC aiguë. Ces médicaments sont recommandés en classe IA dans les guides de pratique.

La fibrose cardiaque est un processus évolutif qui semble être une réponse aux lésions, à l'inflammation ou au stress résultant du vieillissement, des maladies cardiométaboliques telles que l'hypertension, le diabète et les maladies coronariennes. L'évaluation des biomarqueurs protéiques circulants peut informer indirectement sur la fibrose myocardique.

L'approche holistique intègre les données cliniques et d'imagerie avec les "nouvelles" données biologiques (génomiques, protéomiques, métabolomiques) et les sources de données non traditionnelles (base de données en ligne) pour mieux expliquer les systèmes biologiques complexes impliqués dans physiopathologie des maladies. Les technologies émergentes en matière d'acquisition d'images, d'évaluation des données biologiques, telles que la multi-omique, et d'analyse statistique avancée créent un changement de paradigme dans les sciences médicales, permettent une approche holistique. Une approche intégrative comprenant de la protéomique offre un avantage unique pour comprendre l'impact sur plusieurs organes, car les protéines sont les molécules effectrices de l'expression des gènes et des transcrits de l'ARN messager. Par conséquent, une approche intégrative comprenant de la protéomique peut également fournir des indices importants sur la réponse aux thérapies et mieux identifier le rôle de certaines voies impliquées dans la réponse aux médicaments. L'expression des protéines est très dynamique et change en réponse à des modifications de l'environnement, à l'exposition à des médicaments et à la progression de la maladie. En outre, les interactions protéine-protéine peuvent fournir des informations importantes sur les voies physiopathologiques et leurs régulations.

Les réseaux complexes fournissent un cadre permettant d'explorer les relations entre les constituants d'un système biologique de même niveau et de niveaux différents. Dans le champ IC, les réseaux complexes ont identifiés des voies protéiques-biologiques sous-jacentes au développement d'IC. Le système RAAS et les voies fibrotiques expliquent en partie les voies physiopathologiques impliquées dans l'apparition et la progression de la congestion. Plusieurs autres voies liées au stress oxydatif et à l'inflammation ont été peu explorées.

Notre compréhension des mécanismes biologiques de la congestion reste limitée, ce qui entrave le développement de nouveaux biomarqueurs pronostiques et de nouvelles cibles thérapeutiques. Actuellement, la prise en charge de la congestion est essentiellement symptomatique et adaptée à l'état hémodynamique des patients, mais ne tient pas compte de la physiopathologie sous-jacente. Bien que l'inflammation, le stress oxydatif et la fibrose semblent jouer un rôle dans l'aggravation de l'IC, il n'existe pas de médicaments ciblant spécifiquement ces voies. Étant donné l'interconnexion des mécanismes de congestion susmentionnés, des approches holistiques pourraient permettre de mieux comprendre les mécanismes biologiques sous-jacents à la congestion et peut être permettre d'identifier des pistes thérapeutiques.

Hypothèse et objectifs

La congestion a un impact significatif sur les symptômes et la progression de l'IC. Une compréhension plus approfondie de son schéma et de sa distribution peut être utile pour catégoriser les patients atteints d'IC et pour développer des stratégies de gestion personnalisées. Bien que l'évolution clinique de la congestion ait une valeur pronostique et facile à mettre en œuvre, les approches d'apprentissage automatique incluant des données biologiques peuvent identifier des groupes homogènes (profils) de congestion. Cependant pour saisir pleinement la complexité inhérente à l'IC, l'analyse d'un large éventail de variables est nécessaire.

Nous avons émis l'hypothèse suivantes :

- L'intégration des données de congestion cliniques, biologiques et échocardiographiques sur la congestion pourraient permettre de mieux catégoriser l'IC.

- L'évaluation de la congestion pulmonaire au chevet du patient à l'aide de l'échographie pulmonaire peut permettre une meilleure stratification des risques chez les patients atteints d'insuffisance cardiaque, quel que soit leur profil clinique.

- L'ampleur des changements de congestion à l'hôpital, ainsi que l'évaluation combinée de la congestion pulmonaire et systémique, permettent de caractériser plus précisément les patients à haut risque.

- Une approche d'apprentissage automatique non supervisée permet d'identifier, au sein de la population intrinsèquement hétérogène des patients atteints d'IC, des profils de congestion cliniquement significatifs et informatifs.

- Des profils de congestion distincts sont associés à des résultats différents, à des signatures biologiques distinctes, à des mécanismes physiopathologiques différents et pourraient être sensibles à des traitements différents.

Objectifs

1. Evaluer la valeur pronostique en IC :
    a. de la congestion pulmonaire évaluée par la quantification des lignes B par échographie pulmonaire en fonction des profils cliniques et du contexte clinique,
    b. des changements du niveau de congestion à l'hôpital, évalués cliniquement par l'imagerie et par les peptides natriurétiques,
    c. de l'évaluation combinée de la congestion pulmonaire (lignes B) et de la congestion systémique (diamètre de la veine cave inférieure).
2. Identifier et caractériser des profils de congestion distincts dans l'IC en utilisant des approches d'apprentissage automatique non supervisées intégrant des variables de congestion cliniques, biologiques et échocardiographiques et évaluer leur signification pronostique ainsi que les voies biologiques qui leur sont sous-jacentes par l'analyse de réseaux complexes utilisant des données protéomiques.

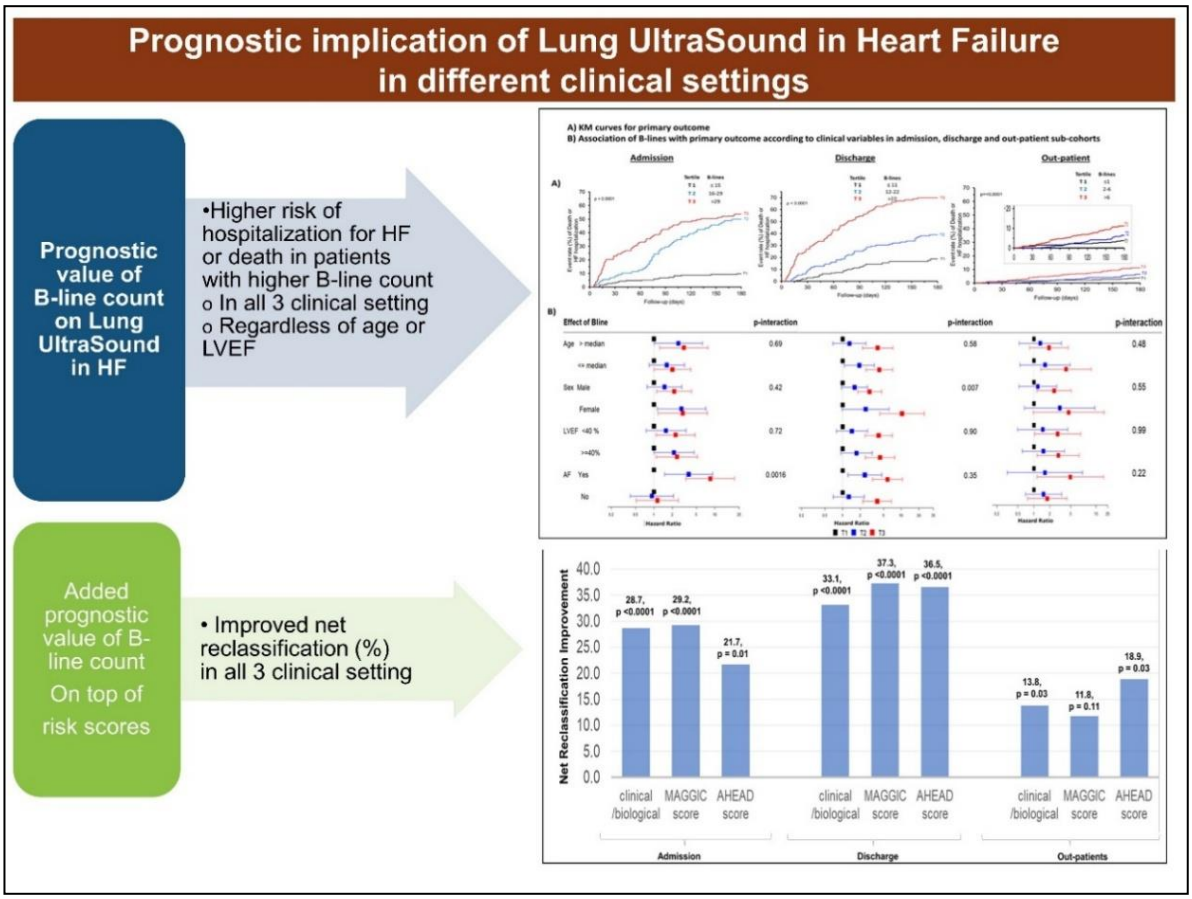
Articles de la thèse

Au cours de cette thèse, j'ai signé en qualité de premier auteur --- trois articles (deux publiés, un en cours de révision), en deuxième auteur un article publié.

La **première partie de cette recherche (1er article)** s'est concentrée sur l'évaluation de l'impact pronostique de la congestion pulmonaire (mesurée à l'aide d'une échographie pulmonaire) dans l'IC. Dans cette étude, nous avons évalué la pertinence pronostique des lignes B dans l'IC dans différents contextes cliniques (à l'admission, à la sortie et en contexte ambulatoire) et selon différents phénotypes cliniques (âge, sexe, fraction d'éjection ventriculaire gauche et présence/absence de fibrillation auriculaire) dans une cohorte internationale de données regroupées (n= 1947). Le protocole d'échographie pulmonaire à huit zones a été utilisé pour calculer le nombre total de lignes B. Nous avons constaté qu'un nombre plus élevé de lignes B était associé à un risque accru de ré-hospitalisation pour IC et de décès toutes causes confondues à la sortie de l'hôpital (Tertile3 vs Tertile1 : HR ajusté= 5,74 (3,26 - 10,12), p<0,0001) et chez les patients ambulatoires (Tertile3 vs Tertile1 : HR ajusté= 2,66 (1,08 - 6,54), p=0,033). L'ajout du nombre de lignes B aux scores de risques cliniques AHEAD et MAGGIC a amélioré la reclassification nette de manière significative dans les trois contextes cliniques (**Graphical abstract 1**).

Les résultats de cette étude montrent que l'échographie pulmonaire fournit des informations pronostiques importantes chez les patients quelle que soit la fraction d'éjection ventriculaire gauche et le contexte clinique. Bien que la capacité pronostique des lignes B ait été influencée par la présence ou l'absence de fibrillation auriculaire dans la cohorte d'admission, les caractéristiques cliniques n'ont pas influencé la capacité pronostique de l'échographie pulmonaire chez les patients ambulatoires. Un résultat important de cette étude est que le nombre plus élevé de lignes B à l'admission n'était pas significativement associé aux prognostics dans les modèles ajustés. Ces résultats suggèrent qu'une décongestion efficace peut être un facteur pronostique plus important que la gravité initiale de la congestion pulmonaire.

Une limitation importante de cette étude est que la capacité pronostique des lignes B a été analysée en évaluant la capacité pronostique à l'admission et à la sortie séparément. De plus, l'évaluation clinique de la congestion à l'aide d'autres scores de congestion ou d'une échographie veineuse n'était pas disponible pour l'analyse.

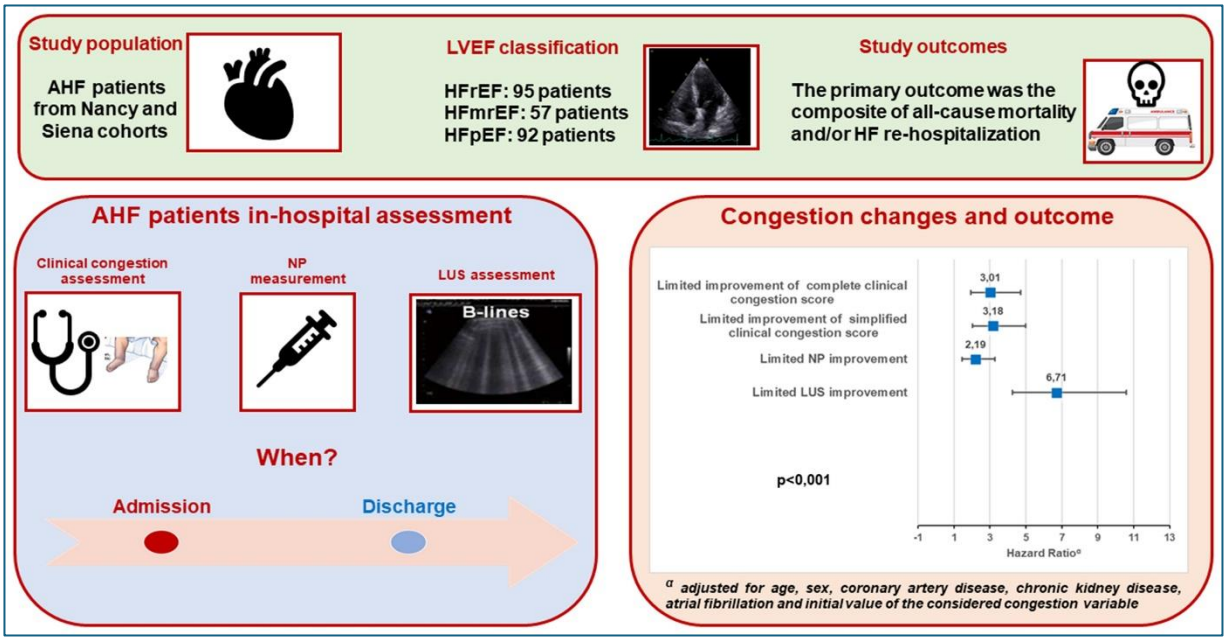


Graphical abstract 1 : article « Prognostic implication of lung ultrasound in heart failure: a pooled analysis of international cohorts » publié dans le revue European Heart Journal – Cardiovascular Imaging.

Pour mieux comprendre le rôle de la congestion pulmonaire sur le devenir des patients, il serait crucial d'évaluer sa résolution en tant que facteur pronostique. La tendance des changements de la congestion, plutôt que les niveaux absolus de congestion, pourrait-être la clé pour optimiser la prise en charge pendant le séjour à l'hôpital.

Dans la deuxième partie de cette thèse, nous avons étudié la pertinence pronostique de l'évaluation combinée de la congestion pulmonaire et de la congestion systémique. Le deuxième article se concentre sur la façon dont les changements de la congestion à l'hôpital entre l'admission et la sortie ont un impact sur le pronostic. En outre, nous avons étudié si une amélioration limitée de la congestion clinique, biologique et pulmonaire à la sortie de l'hôpital était liée à la survenue d'évènements cliniques les sous-types d'IC. Nous avons observé qu'une amélioration limitée/minime des paramètres de congestion tels que le score de congestion clinique (rapport de risque 2,33, IC à 95 % 1,51 - 3,61, p = 0,0001), les niveaux de NP (2,29, IC à 95 % 1,55 - 3,38, p < 0,0001) et le nombre de lignes B (6,44, IC à 95 % 4,19 - 9,89, p < 0,001) au cours de la période de séjour à l'hôpital était associée à un risque accru de mortalité toutes causes confondues et/ou de réhospitalisation pour cause d'IC (**Graphical abstract 2**). Une amélioration limitée du score de congestion clinique et du nombre de lignes B était liée à un mauvais pronostic pour toutes les catégories de fraction d'éjection ventriculaire gauche. Un résultat important de cette étude a été l'observation de taux d'événements cliniques variables parmi les patients qui n'ont montré qu'une amélioration limitée dans diverses combinaisons de paramètres de congestion. Dans cette analyse, nous avons également évalué si le nombre de lignes B variait en fonction de l'état de la fonction rénale dans l'IC. À l'admission et à la sortie, le nombre de lignes B était similaire chez les patients présentant différents états de la fonction rénale (catégorisés comme DFGe<30 ml/min/m^2, 30 à moins de 60 ml/min/m^2, et DFGe≥60 ml/min/$m^{2)}$.

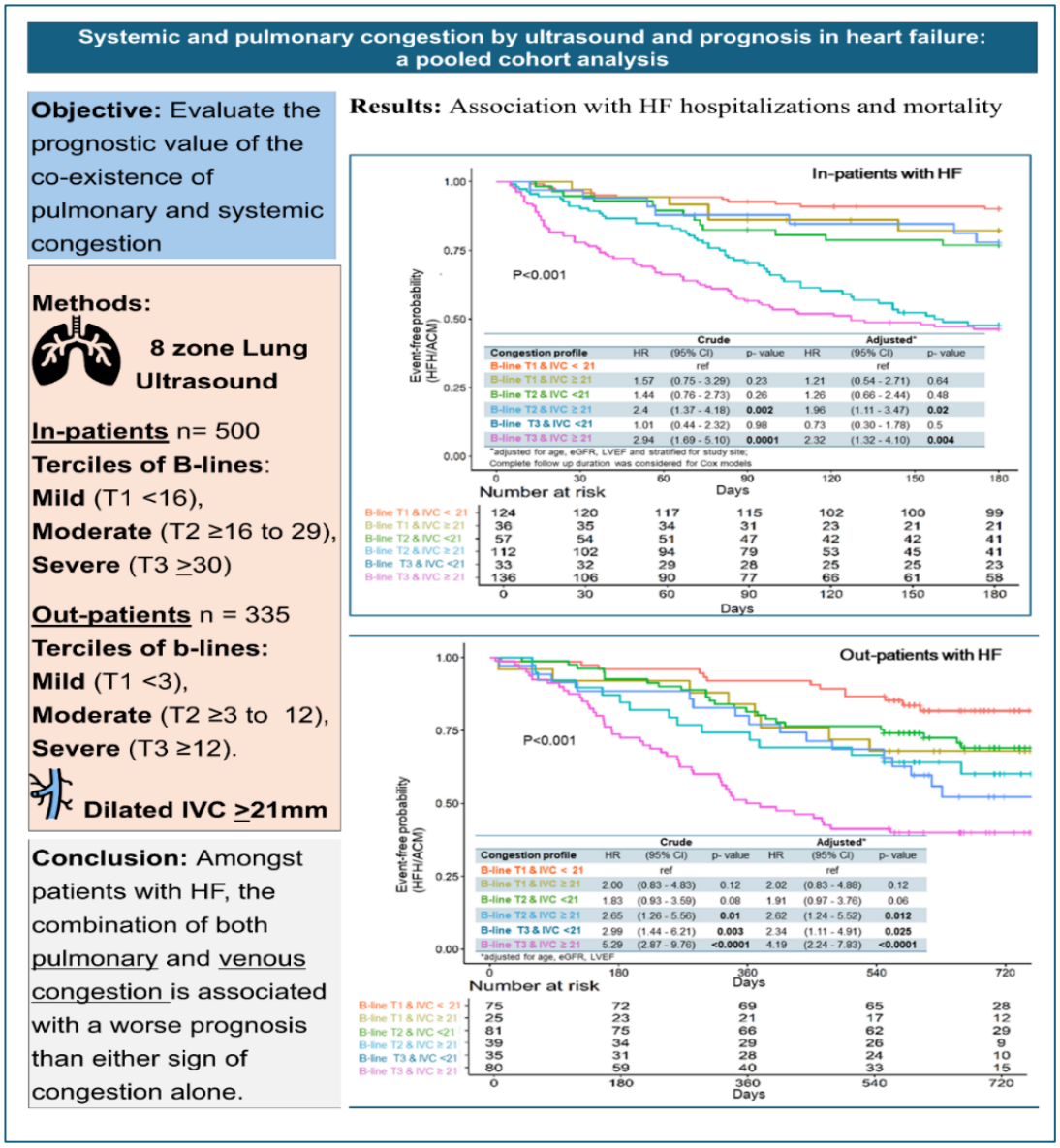
Dans cette étude, nous n'avons pas pu évaluer la congestion veineuse systémique, ce qui est important car les mesures échographiques de la congestion veineuse pulmonaire et systémique ont au mieux une corrélation modérée, ce qui implique qu'elles reflètent différentes dimensions de la congestion. De plus, les différents degrés d'interaction entre la congestion pulmonaire et la congestion systémique pourraient influencer le pronostique. Cela met en évidence d'importantes lacunes dans l'état actuel des connaissances sur la mesure échographique intégrative de la congestion.



Graphical abstract 2: Article « Poor in-hospital congestion improvement in acute heart failure patients classified according to left ventricular ejection fraction: prognostic implications » publié dans la revue European Heart Journal – Cardiovascular Imaging.

Par la suite, **dans le troisième article**, nous avons évalué la valeur pronostique de l'interaction entre la congestion pulmonaire et la congestion systémique dans l'IC en utilisant les données de la cohorte regroupée. Chez les patients atteints d'insuffisance cardiaque, un risque plus élevé a été observé chez les patients hospitalisés comme chez les patients ambulatoires lorsque la congestion systémique coexistait avec une congestion pulmonaire d'intensité au moins modérée. Ces résultats montrent que l'évaluation conjointe de la congestion pulmonaire et systémique améliore la capacité pronostique . Nous avons examiné 835 patients (500 patients ambulatoires et 335 patients hospitalisés) inscrits dans cinq cohortes européennes différentes. Dans cette analyse, nous avons réparti les patients en six groupes en fonction du tercile des lignes B et de l'état de congestion de la veine cave inférieure chez les patients hospitalisés et les patients ambulatoires séparément. Le groupe de référence était constitué de patients présentant une congestion pulmonaire légère (tercile le plus bas des lignes B, <16 pour les patients hospitalisés, <3 pour les patients externes) et une veine cave inférieure non dilatée (<21 mm) (**Graphical abstract 3**). Nos résultats ont mis en évidence l'amélioration de la capacité

pronostique de l'évaluation intégrée de la congestion pulmonaire et systémique. Il a été observé qu'une combinaison de congestion pulmonaire et systémique est associée à un pronostic plus défavorable, alors qu'une congestion systémique et pulmonaire isolée est moins prédictive d'une évolution défavorable. Notre étude fournit des données concrètes qui corroborent la classification conceptuelle de la congestion pulmonaire et systémique en composantes tissulaires/vasculaires et pulmonaires/systémiques.



Graphical abstract 3: Article « Systemic and pulmonary congestion by ultrasound and prognosis in heart failure: a pooled cohort analysis » est en cours de révision.

Dans toutes les études précédentes présentées dans cette thèse, nous avons établi qu'une évaluation plus complète et plus précise de la congestion peut améliorer la stratification du risque. Cependant, dans toutes ces études, la présence de la congestion a été définie à l'aide de seuils prédéterminés. Ces seuils ont été choisis soit sur la base de la littérature publiée, soit par des approches statistiques telles que la constitution de terciles. En outre, nous avons été limités à quelques paramètres de congestion, ce qui altère notre capacité à obtenir une image complète de la congestion.

C'est pourquoi, dans l'article suivant, nous avons choisi d'analyser plusieurs dimensions de la congestion en utilisant des approches d'apprentissage automatique pour identifier les phénotypes de la congestion. En outre, nous avons exploré les fondements biologiques des profils de congestion à l'aide d'un réseau complexe.

Dans la **dernière partie de notre recherche**, nous nous sommes concentrés sur l'identification des profils de congestion à l'aide de l'analyse de clustering. La présente étude visait à élucider la physiopathologie sous-jacente des phénotypes d'IC aiguë basés sur la congestion pulmonaire et systémique à la fois au niveau tissulaire (PTC : congestion tissulaire pulmonaire, STC : congestion tissulaire systémique) et intravasculaire (PIVC : congestion intravasculaire pulmonaire, SIVC : congestion intravasculaire systémique) et à évaluer l'association des phénotypes identifiés avec une critère composite d'hospitalisation liés à l'IC et de décès.

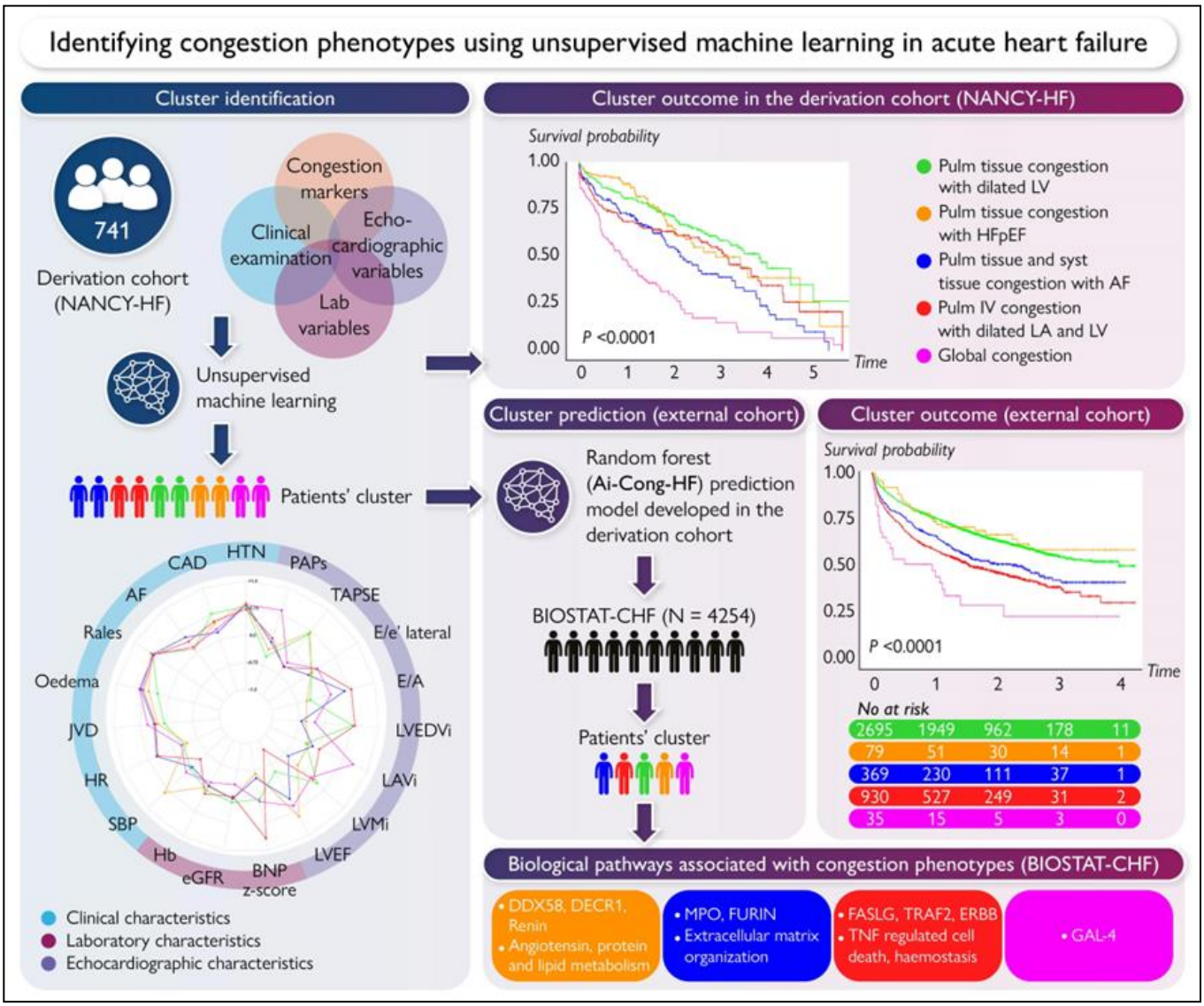
Dix-neuf marqueurs de congestion cliniques, de laboratoire et échocardiographiques ont été analysés à l'aide de techniques de regroupement pour identifier les phénotypes chez les patients présentant une aggravation de l'IC dans la cohorte Nancy-HF (n=741), suivie d'une validation du modèle de clustering dans la cohorte BIOSTAT-CHF (n=4254). Une analyse de réseau a été réalisée à partir de 363 protéines afin d'identifier les voies biologiques sous-jacentes. Cinq phénotypes de congestion ont été identifiés : 1) PTC-dilatation du ventricule (LV), 2) PTC-HFpEF, 3) PTC, STC- fibrillation atriale (AF), 4) PIVC-dilation du l'atrium gauche (LA) and LV and 5) congestion globale. Par rapport au phénotype " PTC- dilatation du LV ", le risque de critère composite était plus élevé dans les phénotypes " PTC, STC-AF " et " Congestion Globale "

[HR ajusté : 1,74 (1,13-2,67) et 2,41 (1,60-3,63), respectivement]. Dans BIOSTAT-CHF, le phénotype de congestion "globale" était associé à un risque significativement plus élevé [HR:1,64 (1,04-2,58)]. Dans l'analyse du réseau, la voie de la réponse immunitaire était liée à tous les phénotypes. "PTC-HFpEF" était lié au métabolisme des lipides, des protéines et de l'angiotensine, "PTC, STC-AF" était lié à la voie de signalisation médiée par les kinases à l'organisation de la matrice extracellulaire et à la mort cellulaire régulée par le TNF, tandis que "PIVC-dilatation LA & LV" était lié à la signalisation médiée par les kinases et à l'hémostase (**Graphical abstract 4**).

Dans l'IC, les techniques de clustering ont permis d'identifier des profils de congestion clinique associés à la fois au risque clinique à long terme et à des différences dans les biomarqueurs, ce qui suggère l'existence de différentes physiopathologies sous-jacentes potentielles. Ces clusters peuvent être appliqués en utilisant le modèle en ligne disponible pour identifier les phénotypes ainsi que les risques associés (https://cic-p-nancy.fr/ai-cong-hf/).

Par rapport aux analyses précédentes menées dans le cadre de cette thèse, la nouveauté de ce travail est qu'il se concentre sur la définition de profils de congestion à l'aide d'un ensemble de marqueurs de congestion. Il s'agit de l'une des premières études à évaluer les profils de congestion dans l'IC à l'aide de modèles basés sur l'apprentissage automatique et à valider le modèle dans une cohorte externe. L'un des principaux défis de ce travail était d'apprendre à effectuer des analyses de regroupement et de trouver des regroupements stables et cliniquement significatifs. En effet, dans l'apprentissage automatique non supervisé, la « vérité » est inconnue, ce qui rend difficile l'interprétation des clusters identifiées. Pour cette analyse, j'ai mené une approche par clustering et plusieurs techniques d'apprentissage automatique supervisé telles que la forêt aléatoire, l'arbre de décision, les modèles eXGBoost pour développer des modèles de prédiction ont été utilisés. En outre, j'ai appris à utiliser des modèles logistiques multinomiaux pour évaluer l'association entre les clusters et les biomarqueurs protéiques.

Dans cette étude, nous avons systématiquement exécuté toutes les étapes du développement d'un modèle validé : identification des clusters, validation interne, développement et comparaison des modèles de prédiction, sélection et validation des modèles de prédiction, et analyse des biomarqueurs.



Graphical abstract 4: article « Identifying congestion phenotypes using unsupervised machine learning in acute heart failure » publié dans la revue European Heart Journal- Digital Health

Nos résultats soulignent l'utilité de l'analyse de clustering en tant que méthode puissante pour identifier des profils homogènes distincts au sein d'une pathologie hétérogène. Les profils se sont révélés très différents dans leurs caractéristiques cliniques et leurs pronostics à long terme. Plus important encore, ils mettent en

évidence la force des algorithmes de clustering pour faciliter une compréhension et une exploration plus approfondies de la physiopathologie sous-jacente complexe.

Discussion et conclusion

Dans ce travail de recherche, nous avons constaté que la congestion pulmonaire évaluée par l'échographie pulmonaire est associée à un pronostic plus défavorable. De plus, le degré de décongestion, quantifié par les changements à l'hôpital du score de congestion clinique, des niveaux de NP et des lignes B, peut améliorer la stratification du risque dans l'IC, indépendamment de la fraction d'éjection ventriculaire gauche. Nous avons également démontré que la combinaison de la congestion pulmonaire et systémique était associée à un moins bon pronostic que l'une ou l'autre congestion prise isolément. Grâce à l'analyse des clusters, nous avons identifié 5 phénotypes distincts de congestion dans l'IC en intégrant divers paramètres de congestion ayant trait aux quatre grands compartiments de la congestion - tissu pulmonaire, tissu systémique, congestion intravasculaire pulmonaire et congestion intravasculaire systémique. Ces phénotypes diffèrent de manière significative dans leurs mécanismes biologiques sous-jacents et leur pronostic. Dans l'ensemble, nos résultats suggèrent qu'une évaluation complète de la congestion est associée à une meilleure évaluation et stratification du risque que si l'on se fie à quelques paramètres de congestion. Une approche basée sur l'analyse des clusters semble prometteuse pour identifier des phénotypes basés sur la congestion dans l'IC. En outre, l'analyse des réseaux de biomarqueurs protéiques a permis de mieux caractériser les voies associées à chaque phénotype de congestion, élargissant ainsi notre compréhension actuelle des mécanismes physiopathologiques sous-jacents.

Les limites de ce projet de recherche sont que toutes les analyses ont été effectuées sur des données de cohortes rétrospectives et que, par conséquent, les résultats doivent être considérés comme générateurs d'hypothèses. En outre, lors de l'analyse de type clustering visant à identifier les profils de congestion, les données telles que les lignes B et la dilatation de la veine cave inférieure n'étaient pas disponibles et, par

conséquent, le tissu pulmonaire et la congestion intravasculaire systémique n'ont pas pu être quantifiés avec précision. En outre, des variables telles que E/e', PAPS et TAPSE n'étaient pas disponibles dans la cohorte BIOSTAT-CHF. Cela aurait pu influencer l'identification des phénotypes de congestion par le modèle de forêt aléatoire.

La force de cette étude réside dans le fait que nous montrons de manière cohérente dans l'analyse de cohortes regroupées que la gravité de congestion ainsi que l'interaction de divers paramètres de congestion influencent le pronostic en IC. Notre analyse des clusters a identifié des profils de congestion distincts, différents en termes de présentation clinique et de pronostic. Le modèle de « random forest » développé pour la prédiction des clusters a démontré une précision de plus de 77%. En outre, nous avons validé notre modèle dans BIOSTAT-CHF, une vaste cohorte externe de patients atteints d'IC, confirmant ainsi l'utilité de notre modèle. À notre connaissance, il s'agit de la première étude qui explore de manière exhaustive les profils de congestion à l'aide d'un large éventail de paramètres de congestion et qui étudie les voies biologiques potentielles liées à chaque profil de congestion.

Nous concluons qu'une évaluation combinée de la congestion pulmonaire, systémique, tissulaire et intravasculaire peut améliorer la stratification du risque chez les patients souffrant d'IC aiguë et chronique. Les modèles pilotés par l'IA sont utiles pour identifier des profils de congestion homogènes au sein de populations d'IC hétérogènes. Ces profils diffèrent en termes de pronostic à long terme et de mécanismes sous-jacents. Le phénotypage des patients en fonction de leur profil de congestion pourrait s'avérer utile dans des études ultérieures évaluant de nouvelles approches thérapeutiques de l'IC. Cependant, des recherches supplémentaires intégrant toutes les dimensions de congestion notamment via des données échographiques et biomarqueurs avec une grande rigueur méthodologique sont nécessaires avant que les modèles pilotés par l'IA puissent être intégrés dans la pratique clinique de routine ainsi que la création de liens entre les modèles et les dossiers patients.

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List of abbreviations

ACEi	Angiotensin Converting Enzyme inhibitor
AF	Atrial Fibrillation
AHF	Acute Heart Failure
ARA	Angiotensin Receptor Antagonist
ARNi	Angiotensin and Neprilysin Inhibitor
BMI	Body Mass Index
BNP	Brain Natriuretic Peptide
CAD	Coronary Artery Disease
COPD	Chronic Obstructive Pulmonary Disease
CV	CardioVascular
eGFR	estimated Glomerular Filtration Rate
FDR	False Discovery Rate
GDMT	Guideline Directed Medical Therapy
Hb	Hemoglobin
HF	Heart Failure
HFmrEF	HF with mildly-reduced Ejection Fraction
HFpEF	HF with preserved Ejection Fraction
HFrEF	HF with reduced Ejection Fraction
HR	Heart Rate
HTN	Hypertension
JVD	Jugular Venous Distention
JVP	Jugular venous Pressure
LA	Left Atrium
LAVi	Left Atrial Volume index
LCM	Latent class model
LOD	Limit Of Detection
LUS	Lung UltraSound
LV	Left Ventricle
LVEDVi	Left Ventricular End Diastolic Volume index
LVEF	Left Ventricular Ejection Fraction
LVMi	Left Ventricular Mass index
MRA	Mineralocorticoid Receptor Antagonist
NP	Natriuretic Peptides
NYHA	New York Heart Association
PASP	Pulmonary artery systolic pressure
SBP	Systolic Blood Pressure
TAPSE	Tricuspid Annular Plane Systolic Excursion
Pulm	Pulmonary
RF	Random Forest
SGLT2i	Sodium Glucose Cotransporter 2 inhibitor
XGBoost	eXtreme Gradient Boosting

1. Introduction

Congestion is one of the main drivers of symptoms and hospitalization in heart failure (HF) and is associated with poor prognosis [1,2]. Congestion is related to increased pulmonary or systemic pressures which results from and contribute towards volume overload resulting due to sodium-water retention [3]. Accurate congestion assessment can provide valuable information on the risk of adverse outcomes, affected organs, disease severity and progression. It can guide treatment decisions and is useful in monitoring treatment response.

Congestion evaluation tools include physical examination, natriuretic peptides and imaging such as echocardiography, chest X-ray and lung ultrasound (LUS). LUS provides a quick, semi-quantitative and accurate measure of lung congestion by measuring B-lines, a comet-like artifact seen due to presence of fluid in interstitial lung tissue. LUS findings can corroborate clinical findings to confirm HF diagnosis in patients with acute dyspnea / suspected HF. B-lines have been used to guide diuretic doses and stratify risk in patients with HF. While data regarding clinical relevance of clinical congestion scores and natriuretic peptides is well-established, more evidence is required to firmly establish the utility of LUS as prognostic marker. Most of the published studies are limited by small sample size, focus on either in-patients or out-patients, and varied different LUS protocols. Also, it is unclear if LUS findings remain prognostically relevant in different LVEF categories and comorbidity profile in HF. In addition, it needs to be determined whether the congestion changes measured by integrating LUS findings in conjunction with clinical congestion scores and natriuretic peptide levels improve risk stratification. Beyond the prognostic relevance of pulmonary congestion quantification by LUS, its clinical significance in presence of other congestion variables has been scarcely investigated.

At present, HF is classified according to NYHA functional classification, arbitrary left ventricular ejection fraction (LVEF) cut-offs and A to D stages to describe disease severity and recommend management plans [4-6]. Another approach centered on congestion and cardiac output has been promoted

in recent European guidelines, based on the Forrester classification of “wet/dry” and “cold/warm” phenotypes; however, this approach does not precisely reflect congestion distribution and pattern [7]. Given the central role of congestion in HF pathophysiology and symptom load, identifying congestion based profiles would be relevant in classifying HF patients and possibly designing management plan.

Recently, conceptual framework classifying congestion into pulmonary, systemic, intravascular and tissue congestion have been proposed to identify predominant congestion profile. This framework accommodates the diverse mechanisms of congestion. In addition, it is important to note that most of the patients have an overlap of these congestion types [3,8]. Hence, since this framework is eminence-driven, adopting a data-driven and/or agnostic approach to congestion variables would help provide objective evidence to support—or challenge—its validity. Moreover, how congestion patterns impact the prognosis, treatment plan and risk stratification remain unclear. In addition, complexity of congestion and its interaction with other factors may not be captured by conventional approaches that classify congestion based on pre-determined cut-offs. Given the heterogeneity of congestion variables, artificial intelligence-driven models integrate multiple congestion parameters such as clinical sign and symptoms of congestion, imaging markers and protein biomarkers which could ultimately unravel evidence-based congestion profiles.

Congestion biomarkers such as natriuretic peptides, adrenomedullin, bioADM, sST2, CD 125 have been extensively studied for their role in diagnosis and management in HF [9]. Natriuretic peptides are more indicative of intravascular congestion whereas, bioADM and CD 125 are more suggestive of tissue congestion. In addition to these established biomarkers, research indicates that congestion can trigger a cascade of neuro-hormonal, inflammatory, and fibrosis protein-pathways, with specific pathways varying based on patient profile and congestion severity. Nevertheless, data regarding the biological pathways linked to congestion profiles is currently limited, and the nature of protein pathway interactions is not

yet fully understood. This could typically be done using complex networks leveraging on circulating protein data to explore protein-pathways associated with congestion phenotypes [10,11].

Overall, despite advances in congestion assessment methods and analytical approaches, significant gaps remain regarding the prognostic value of integrated strategies that include imaging modalities such as lung and venous ultrasound—particularly when using conventional congestion cut-offs for stratification. Moreover, further evidence is needed to evaluate the clinical utility of novel agnostic approaches for identifying evidence-based congestion profiles or for patient stratification in a clinically meaningful way. Finally, combining proteomic analyses with network science may offer new insights into the biological mechanisms underlying distinct congestion patterns.

Hence, in this thesis we investigated the prognostic association of 1) number of B-lines in patients with different clinical profiles and in clinical setting, 2) changes in clinical congestion score, B-lines and natriuretic peptide on prognosis 3) combined assessment of pulmonary (B-lines) and systemic congestion (IVC diameter). Further, we identified congestion phenotypes using clustering approaches on data consisting of comprehensive array of clinical, biological and echocardiographic congestion markers, indicating pulmonary, systemic, venous and/or tissue congestion. We also explored the complex pathophysiology of congestion by conducting network analysis on circulating protein data to identify biological pathways associated with specific congestion patterns.

2. Context

2.1. Disease burden of HF and its risk factors

HF carries a substantial disease burden because of high morbimortality, decreased quality of life, frequent hospitalization and health care costs. **The global prevalence of HF is estimated to be between 1 and 3%** (approximately 56.2 million individuals) [12]. However, there is considerable heterogeneity between different countries and geographic zones. Within France, there are more than 1.3 million prevalent cases of HF, which is about 2.6% of its adult population [13]. In 2022, the crude rate of hospitalization was 339 per 100,000 inhabitants and 34.0% patients hospitalized for HF died within a year of hospitalization. Some departments of Grand Est region of France, had higher age-standardized rates of patients hospitalized for HF which is likely due the higher prevalence of risk factors such as diabetes and obesity.

Despite newer therapeutic agents and better management protocols, a trend of increased incidence of HF has been seen over the last decade [12] which reflects the ageing population and increased incidence of cardiovascular and metabolic diseases. The well-established and common risk factors for HF are older age, myocardial infarction, hypertension, diabetes, obesity, congenital cardiomyopathy, valve disorder, arrhythmia, smoking among others. Importantly, risk factors such as atrial fibrillation, renal function and BMI often impact accuracy of congestion assessment.

2.2. Congestion: Cornerstone of HF definition and its impact on prognosis

HF is a complex clinical syndrome consisting of clinical signs and symptoms such as difficulty in breathing, ankle swelling, elevated jugular venous pressure, rales and peripheral oedema and elevated natriuretic peptides; however, there is no universally accepted definition of HF [6,14]. The structural and

functional abnormalities in HF cause increased filling pressures and inadequate cardiac output leading to salt-water retention and organ congestion.

The signs and symptoms of congestion are not only the key criteria to diagnose HF but one of the primary reasons for hospitalization. Hence, de-congestion is one the principal therapeutic goals in acute HF. However, **complete decongestion in patients with HF remains challenging despite treatment with diuretics and other HF therapies such as SGLT2i, ARNi and MRAs** [2,15]. Studies show that 40 to 50% patients are discharged with residual congestion when assessed with LUS. Notably, residual congestion was associated with poorer outcome compared to the "dry" patients at discharge [16,17]. Moreover, signs and symptoms of congestion were associated with the increased 30-day adjusted risk of death by14% for jugular vein distention and 96% for dyspnea on exertion [18]. Similarly, dilated IVC was associated with higher risk of re-hospitalization over a 60- day follow up in acute HF [19]. However, congestion is very heterogenous in its distribution and severity. **Further, severity of congestion does not necessarily reflect severity of HF and its role in disease progression is still not clear.** A better understanding of congestion mechanism and congestion profile could be useful in stratifying risk in addition to personalizing treatment approach.

2.3. Mechanism / pathophysiology of congestion in heart failure

Conventional understanding considers that congestion is a homogenous uni-compartmental accumulation of fluids in extravascular spaces causing tissue oedema. Cardiac injury/myocardial dysfunction results in venous and capillary stasis which disturbs the starling forces and thereby altering microcirculatory hemodynamics [20]. Changes in hydrostatic and oncotic forces lead to fluid shift from intravascular space to the interstitial space contributing towards reduced effective circulating volume in HF. In addition, insufficient blood supply to the organs including kidneys, which activates renal sympathetic system further deteriorating the congestive symptoms (discussed in detail in the section

2.6). **Reduced effective circulating volume and decreased renal artery circulation often labelled as "arterial under-filling" trigger compensatory response of salt – water retention to increase plasma volume** [21].

Other important mechanisms which regulate plasma volume are the neurohormonal and baroreceptor reflex pathways [21-23]. The heart synthesizes natriuretic peptides such as atrial natriuretic peptides and brain natriuretic peptides in response to myocardial stretch because of increased filling pressures. **These peptides produce natriuresis and diuresis, thus reducing plasma volume. Concurrently, baroreceptor reflex activated by increased filling pressures suppress neurohormonal system**, further regulating fluid balance. Though these responses may be blunted in patients with HF [21].

However, **current clinical consensus supports a more dynamic model that acknowledges its multi-factorial origin, complexity of fluid redistribution, and its clinical manifestations.** Congestion is **influenced by interaction of several factors such as cardiac and renal function, integrity of endothelial membranes, role of interstitial and extravascular compartments impact signs congestion profile.** Moreover, even in the absence of plasma volume expansion, congestive symptoms may occur due to fluid redistribution from different compartments such as splanchnic system to vascular system [22,24].

In light of the multi-factorial regulation of fluid volume, inherent heterogeneity in the venous capacitance at different organ level and patient to patient variability, it would be important to understand the different patterns of fluid distribution in vascular and extravascular compartments as well as by differences in the fluid accumulation in systemic and pulmonary levels.

2.3.1. Tissue congestion vs intravascular congestion

Although, **tissue and intravascular congestion are inter-linked phenomenon, there are important distinctions in pathophysiology and hence, differences in management approach (Figure 1 and Figure 2).** Fluid accumulation in the intravascular compartment results from renal salt water retention in response to "arterial-underfilling". On the other hand, fluid redistribution in absence of fluid accumulation can also trigger acute decompensation even in absence of systemic or venous congestion. Sudden displacement of fluid from systemic circulation to pulmonary circulation due to transient vasoconstriction can cause pulmonary congestion even in absence of systemic fluid retention or accumulation [25]. Patients with acute HF often present with sudden or progressive increase in cardiac and pulmonary filling pressures without overall systemic congestion. In patients with fluid accumulation diuretics are effective in decreasing intravascular volume [26], whereas patients with hypertensive / redistributive HF respond well to vasodilator therapy [27,28].

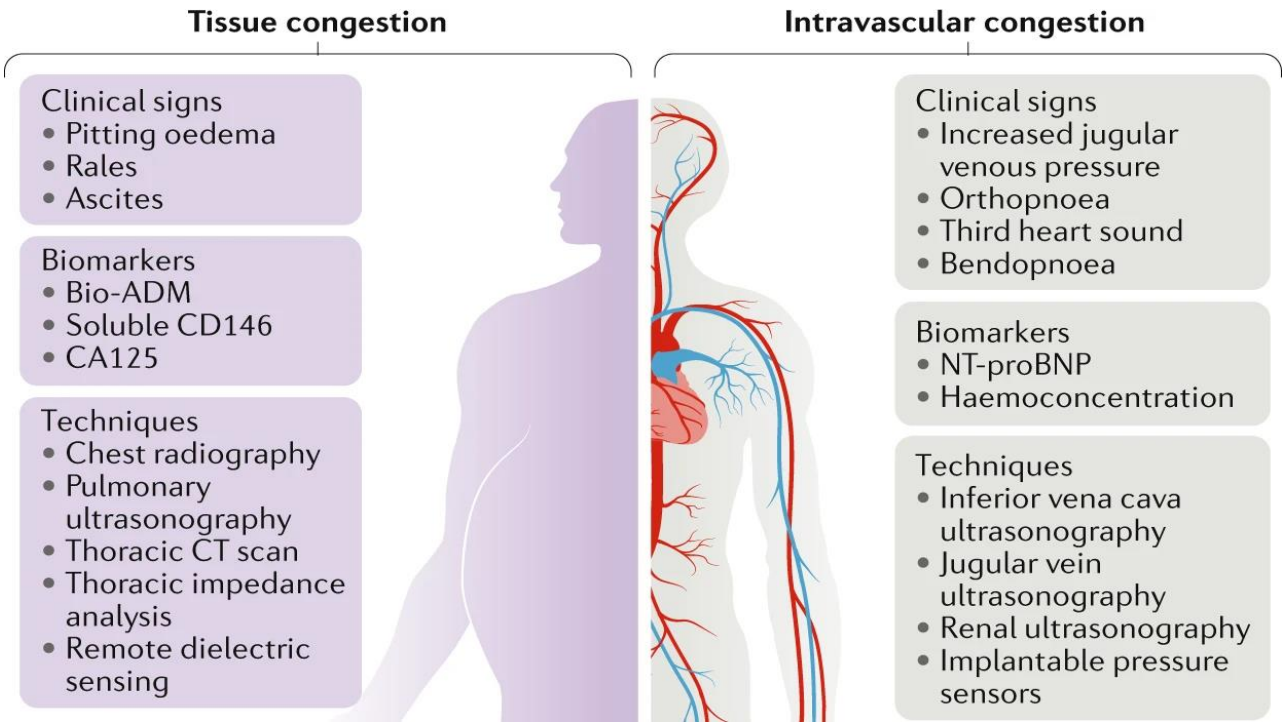

Figure 1: Hypothesized differences between tissue congestion and intravascular congestion. (from Boorsma et al., [8])

Progressive and sustained increase in fluid in vascular compartment leads to transcapillary leaks, shifting the sodium and fluid from vascular compartments to extravascular spaces. In the interstitial space, glycosaminoglycan networks made of proteoglycan chains provide structure to the interstitium by forming gel-like structure. These networks bind the water molecules present in the interstitial space. The proteoglycans are negatively charged and therefore, can bind sodium ions. Prolonged sodium accumulation due to increased hydrostatic pressures alters the structural and functional integrity of glycosaminoglycan networks which reduce the buffering capacity of interstitial space, causing pulmonary or peripheral oedema with slight increase in water retention [8,29]. Moreover, sodium associated with glycosaminoglycans does not reach kidneys and hence, escapes diuretic therapy leading to residual or sustained oedema.

2.3.2. Systemic vs pulmonary congestion

Increase in left heart filling pressure is reflected as raised pressure in pulmonary veins and capillaries. The raised capillary pressures overcomes the colloid osmotic pressure in the perialveolar interstitium causing fluid accumulation in the alveoli [30]. Although lymphatic adaptations in the lungs increases interstitial fluid drainage, elevated interstitial pressure hinders the lymphatic drainage, further aggravating pleural effusion and pulmonary oedema [20]. Repeated or severe pulmonary oedema can cause cardiopulmonary remodeling such as endothelial dysfunction, fibrosis and thickening of extracellular matrix which can impair alveolar gas exchange causing difficulty in breathing. Other signs and symptoms of pulmonary congestion include rales, crackles, B-lines on lung ultrasound and elevated pulmonary artery pressure on echocardiography.

On the other hand, systemic congestion reflects the increased pressure in the right heart. Increased filling pressures of the right heart are associated with dilated IVC. Venous congestion has been reported to be associated with worsening of renal function in patients with advanced HF [31]. In addition, systemic

congestion extends to hepatic and intestinal congestion. Several patients with acute HF present with cholestasis and raised liver enzymes. Hepatic dysfunction is linked with worse prognostic outcomes in patients with acute HF [32]. Systemic congestion also contributes towards interstitial congestion in the gut which alters the gut permeability thereby causing absorption of bowel toxins and gut-microbioata [33]. These effects can trigger inflammatory response which further aggravates the cardiac dysfunction [33]. Patients with systemic congestion often have raised jugular venous pressure, hepatomegaly and abdominal swelling.

While most of the patients with HF have some features of both pulmonary and systemic vascular congestion, one type of congestion may be more prominent depending on patient susceptibility and venous capacitance [34,35]. Moreover, diagnostic and therapeutic strategies need adaptation depending on the congestion subtype and organs involved (**Figure 2**).

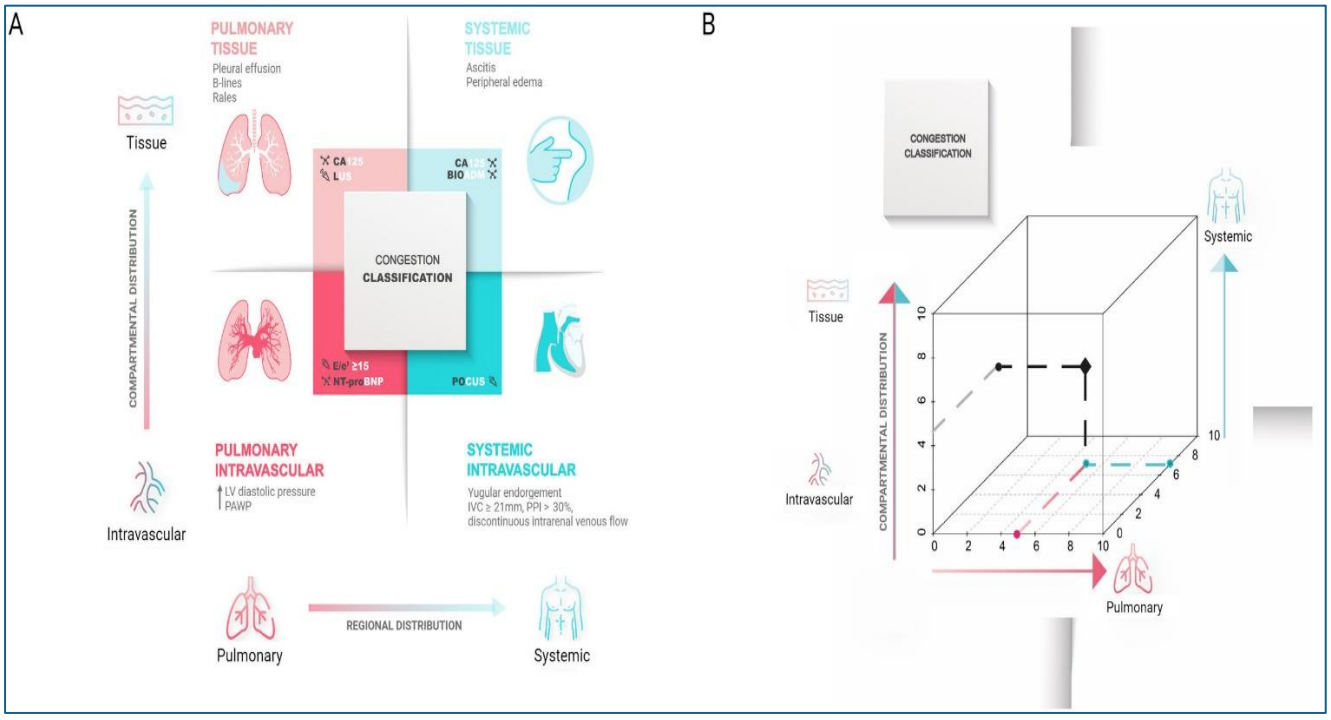

Figure 2: 2-dimensional (A) and 3-dimensional (B) regional and compartmental distribution of congestion by physical examination, circulating biomarkers, and imaging techniques. (from de la Espriella et al., [3])

2.4. Congestion markers and congestion classification commonly used in clinics

2.4.1. Congestion markers on physical examination

2.4.1.1. Pulmonary rales

Presence of rales on auscultation may indicate fluid in pulmonary interstitial spaces. Presence of rales is associated with increased risk of odds of 28-day mortality in patients with HFrEF and HFpEF in patients admitted for acute HF in the ARIC study [36]. Rales was an independent prognostic marker of cardiovascular mortality, all-cause mortality and HF hospitalization in the AF-CHF Trial (Atrial Fibrillation and Chronic Heart Failure) [37]. However, rales is not a specific sign of HF and is also found in patients with other pulmonary diseases such as, pulmonary infection, cancer or fibrosis.

2.4.1.2. Leg oedema

Leg oedema is associated with increased tissue fluid overload in HF. Peripheral edema usually develops over a prolonged period of time and is due to gradual and progressive deterioration of heart function [38]. A longer treatment period with predominantly diuretics is required to improve symptoms of peripheral oedema. A study in a large pooled cohort of HFpEF patients reported that patients with higher degree of peripheral oedema had higher jugular venous distention suggesting raised intravascular volumes [39]. Presence of leg oedema was independently associated with increased risk of cardiovascular mortality in patients enrolled in AF-CHF Trial [37]. Similar findings were reported in ARIC-HF study wherein leg oedema was associated with increased risk of cardiovascular mortality and all-cause mortality [36]. A study by Shoaib et al., showed that severity of leg edema (classified as none, mild, moderate or severe) was associated with both length of hospital stay and mortality during index admission and after discharge[38]. The hazard ratio for death was 1.51 (CI 1.50–1.53) for severe, 1.21 (CI 1.20–1.22) for moderate and 1.04 (CI 1.02–1.05) for mild peripheral oedema compared to patients without peripheral oedema at presentation. However, degree of peripheral oedema does not necessarily

correlate with the symptom burden in HF [40]. Moreover, leg oedema is a non-specific sign and can be elevated in conditions like hypoalbuminemia and nephrotic syndrome.

2.4.1.3. Dyspnea, Orthopnea and Bendopnea

Dyspnea is a classical sign of HF and one of the most common presenting symptoms in acute HF. Patients may present with different types of dyspnea and dyspnea severity. Patient-reported dyspnea at rest has been linked with worse prognosis in Acute Decompensated Heart Failure National Registry (ADHERE) [41]. However, in the post hoc analysis of EVEREST trial, physician reported and patients reported dyspnea was not associated with health-related quality of life, all-cause mortality, or all-cause or cause-specific hospitalization [42]. Orthopnea or difficulty in breathing in supine position was associated with pulmonary capillary wedge pressure ≥30 mm Hg in the ESCAPE trial [43]. However, dyspnea or orthopnea can be of non – cardiac origin.

Bendopnea, a type of dyspnea, occurs when patients bend forwards while sitting in their chair and try to touch their feet. This sign was described in patients with advanced HF and was associated with increased filling pressures [44]. This sign has been observed in 18 to 49% of HF patients including ambulatory patients. In a recent study by de la Espriella et al., it was shown that a reduction of 3% in oxygen saturation on bending (but not bendopnea) was associated with higher risk of worsening HF [45].

2.4.1.4. Jugular venous distention

Jugular venous distention (JVD) reflects the right atrial pressure and PCWP and is considered a more specific sign than dyspnea or edema in HF. In an observational study, presence of JVD at index hospitalization was associated with increased risk of 10-year mortality, with a more pronounced effect in younger patients [46]. In a study which assessed jugular venous pressure (JVP) before and after 6-minutes' walk test found that the patients who had high JVP had a higher risk of CV mortality and HF hospitalization [47]. Compared to other physical signs congestion, raised JVP in particular was reported to have worse prognostic outcome in multicenter acute HF registry [48]. Precise measurement of JVD is

difficult and may be affected by patient habitus and respiratory pathology [1]. JVD measurement is particularly difficult in obese patients and lacks reproducibility.

2.4.2. Clinical congestion score

While individual congestion markers provide important information on congestion severity and pattern, their prognostic performance is limited [1]. **However, clinical congestion scores which combine clinical signs and symptoms offer more accurate assessment of congestion severity and better predict patient outcomes.** Subsequent congestion scores can be used to assess response to therapy in congested patients.

In 2010, Gheorghiade et al., proposed a systemic approach to grade congestion by scoring commonly available congestion measurements [1]. The components of this score were: beside assessment of congestion (orthopnea, JVD, edema, hepatomegaly), natriuretic peptide (NP) levels and dynamic maneuvers (orthostatic testing, 6-minute walk test and Valsalva maneuver). Each component was given a score according to the degree of severity. However, assessing this score in routine clinical examination is challenging as time constraints and patient's condition limits data collection. Later, Ambrosy et al., evaluated a congestion score consisting of congestive signs and symptoms in a post hoc analysis of EVEREST trial [49]. This analysis included 2061 patients with HFrEF who were in the placebo group of EVEREST trial. The congestion score consisted of dyspnea, orthopnea, fatigue, rales, pedal oedema, and JVD and rated signs and symptoms on a standardized 4-point scale ranging from 0 to 3. This study showed that per unit increase in combined congestion score at discharge was associated with increased risk of hospitalization and/or ACM over a period 30 days as well as over the duration of study (~ 9.9 months). However, it should be noted that associations were relatively small with a hazard ratio of 1.13 (1.03, 1.25) for 30-day follow-up period and 1.11 (1.06, 1.17) for overall duration of study. Among the patients having congestion score 3-9, 60 % patients had an outcome of ACM and Hospitalization for HF, whereas 36% patients having congestion score 0 had an outcome. An important finding of this study was

that **though NP levels declined concurrently with clinical signs and symptoms, the levels of NP (both BNP and NTproBNP) remained markedly elevated at the time of discharge suggesting that clinical sign and symptom changes do not adequately capture the hemodynamic congestion status.** These findings indicate that combined congestion score consisting of diverse congestion markers such as clinical signs and symptoms, NP and findings from bedside ultrasound which are readily available may assess congestion more accurately and reliably.

2.4.3. Congestion markers on ultrasound

2.4.3.1. Transthoracic echocardiography

Echocardiography is a key investigation for diagnosing HF and establishing a treatment plan [5,50,51]. Echocardiography provides information on LVEF, cardiac structure (eg. LV mass, volume, interseptal thickness), systolic and diastolic function (eg. LV end systolic and diastolic volumes, LAVi) as well as on estimated pulmonary artery pressure and RV function. HF is categorized into HFrEF, HFpEF and HFmrEF. **Echocardiographic parameters such as LAVi and E/e' been shown to be independent prognostic markers in HF** [52-55]. Echocardiographic parameters play a crucial role in diagnosis and risk stratification in patients with HFpEF [56,57]. In addition, echocardiography provides details on possible underlying causes such as valve disease or myocarditis.

However, **diagnostic accuracy of individual echocardiography parameters to diagnose HF is limited, especially in HFpEF**. In Euro-filling study, it was shown that parameters such as E/A, E/e', TRV and LAVi have a poor to modest correlation with invasive LV end diastolic pressure (0.46, 0.34, -0.15 and 0.28, respectively) [58]. Based on the available data in this study, the accuracy of this algorithm for estimating raised LV filling pressure is 55.9% in comparison to invasive LV filling pressure estimation (89 patients correctly identified vs 132 wrongly identified + 27 indeterminate) . Moreover, a systematic review reported that pooled correlation coefficient of E/e' with invasively measured LV filling pressure

was 0.56 [55]. In 2016, the American Society of Echocardiography (ASE) in association with the European Association of Cardiovascular Imaging (EACVI) proposed an algorithmic approach to estimate LV diastolic dysfunction using echocardiographic parameters in patients with normal LVEF [59] (**Figure 3**). The algorithmic approach to estimate LV filling pressure combines 4 echocardiographic parameters, E/A, E/e', TRV and LAVi. However, this approach has limited sensitivity (average = 61%) and varies from 34% to 87% [60] and a sizeable proportion of patients are characterized as 'indeterminate' [55]. Moreover, this algorithm does not take into account echocardiographic parameters measured during exercise to estimate filling pressure and cardiac structural abnormalities like LV hypertrophy. Further, in obese individuals LAVi may be low and hence, leading to erroneous conclusion [61].

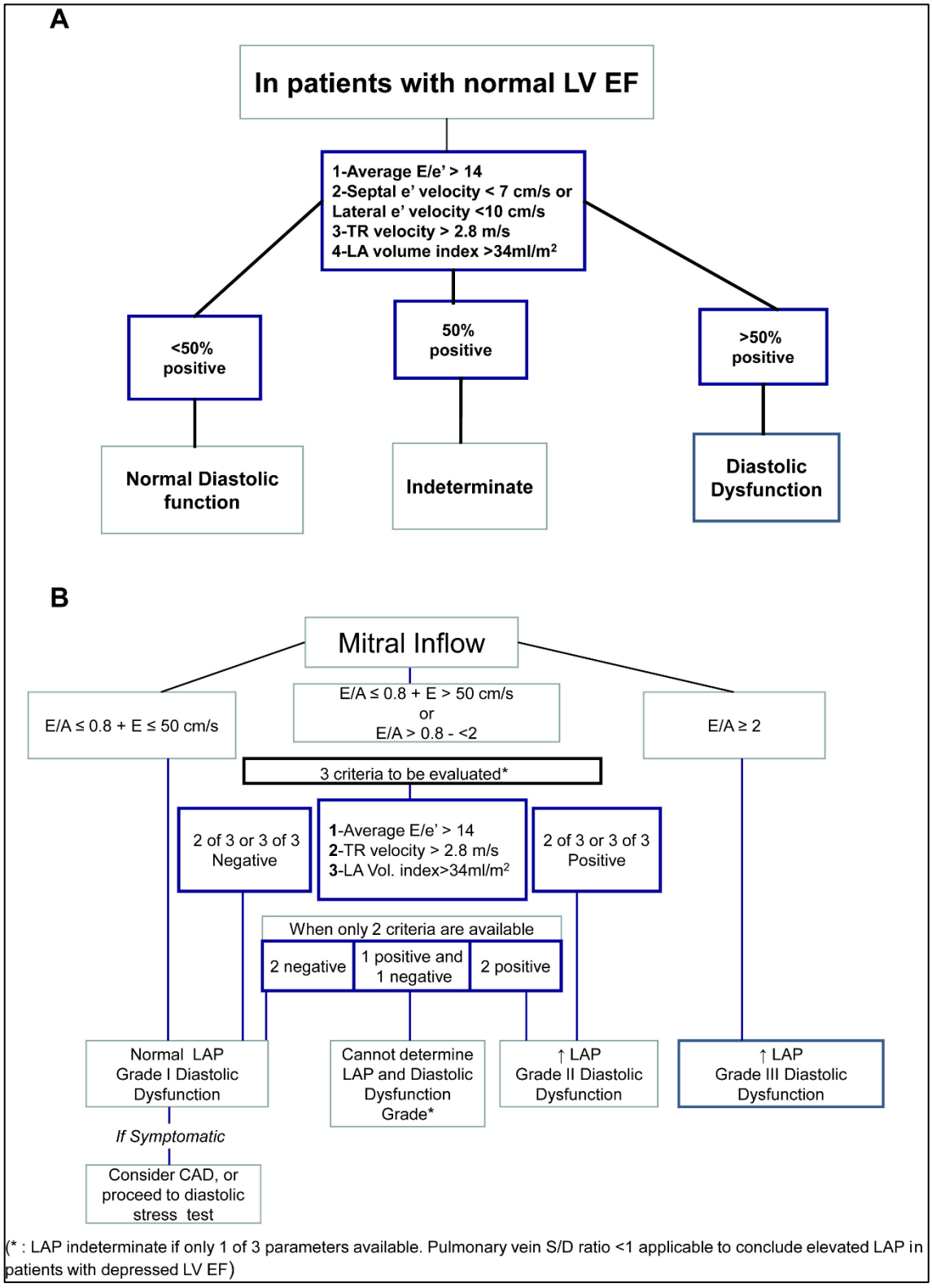

Figure 3: Noninvasive Assessment of LV diastolic dysfunction in subjects with normal LVEF (from Nagueh et al;[59])

2.4.3.2. Lung ultrasound

LUS is a semi-quantitative method to measure pulmonary congestion [62]. The assessment is based on detection of B-lines, also known as "comet-tail artifacts which appear as laser-like artifact on ultrasound. B-lines originate from presence of extravascular fluid in the lungs. The primary advantage of LUS is its easy integration into cardiology/ out-patient clinics, especially with the availability of hand-held devices, to identify subclinical congestion. Various scanning protocols of LUS have been reported in previous studies which include 4, 6, 8 or 28 -zone scanning protocol to identify and quantify congestion in patients with HF (**Figure 4**). Presently, 8-zone scanning LUS protocol is most widely used because it is both rapid and accurate for diagnosis, risk stratification and titrating diuretic doses [62].

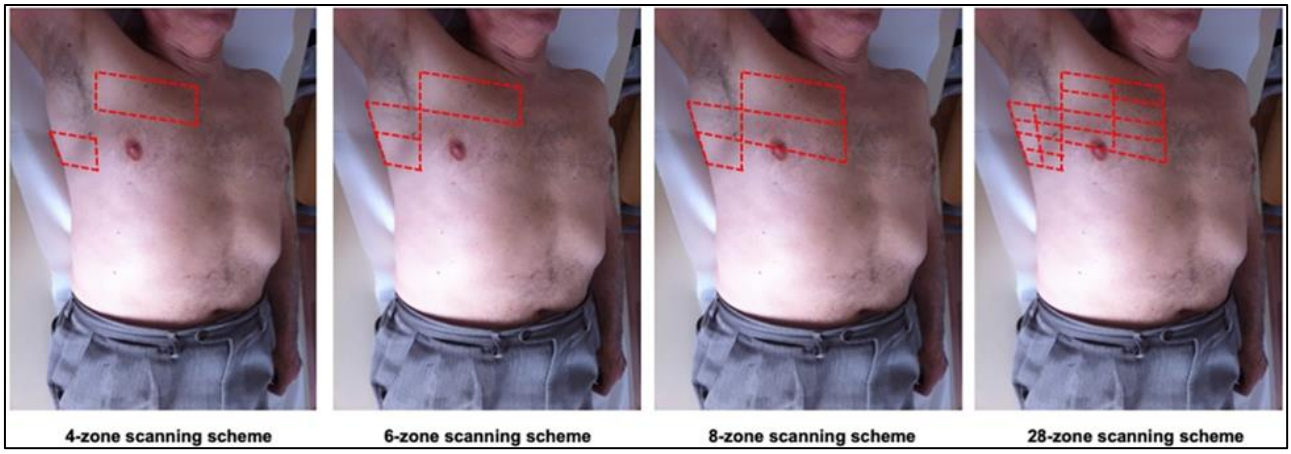

Figure 4: Different LUS scanning protocols, ranging from 4 to 28 zones (from Gargani et al., [62])

In emergency departments, LUS is particularly useful in patients presenting with acute dyspnea especially when there is diagnostic uncertainty [63]. The 2021 ESC guidelines suggest using LUS in acute settings, particularly when NTproBNP measurements are not available, to confirm HF diagnosis [5]. In suspected HFpEF, cardiac catheterization can provide direct measures of filling pressures and aid in confirming the diagnosis. However, bedside non-invasive echocardiographic assessment remains the

cornerstone for diagnosing HF. Recent studies have shown that number of B-lines had a moderate correlation with right atrial (r = 0.32), pulmonary artery diastolic (r = 0.34), mean pulmonary artery (r = 0.43) pressures and pulmonary vascular resistance (r = 0.51) [64] measured using right heart catheterization. However, it should be noted that this study population (n= 92) had large number of patients without acute HF as well as patients with post cardiac transplant. The pulmonary physiology and susceptibility to pulmonary edema in presence of elevated filling pressure may vary among the included subjects. Another study showed that B-line count had better correlation (r = 0.62) than E/A (r = 0.34) and IVC (r = 0.38) with LVEDP measured using left heart catheterization. This study also showed that addition of B-line count on top of clinical variables improve the diagnostic accuracy of elevated LVEDP [C-index = 95.5 (90 to 100)] and improved net reclassification index by 145.4 (113.0 to 177.9) [65]. Moreover, the improvement in diagnostic accuracy with B-lines was much higher than any of the left-sided echocardiographic parameters to assess LV filling pressures. Although IVC diameter significantly increased the diagnostic accuracy for filling pressures. These findings indicate that systematically incorporating LUS findings to conventional echocardiography can improve diagnostic accuracy of identifying pulmonary congestion.

In patients with HF, a high number of B-lines is associated with worse prognostic outcome in both ambulatory and hospitalized patients [35,66,67]. In the wet-to-dry HF study, it was reported that patients with global HF (B-lines: 20.6 ± 11) decompensation had the highest number of B-lines, followed by left-sided (19.7 ± 11.6) and right-sided (13.5 ± 9.8) HF [68]. Moreover, increase in 1 B-line was associated with 4% increased risk of all-cause death or HF-related hospitalization in outpatients. In a proof of concepts study by Rivas-Lasarte et al., it was demonstrated that LUS-guided treatment reduced the risk of combined endpoint (rehospitalization, unexpected visit for HF worsening or death at 6-month follow-up) in acute HF patients (HR: 0.518 [95%CI 0.268–0.998; P = 0.049] and improved their walking capacity[69]. The number of B-lines changes rapidly in response to diuretics therapy and therefore serial LUS can be used for monitoring the response to diuretics therapy [70]. A meta-analysis on prognostic and therapeutic utility

of LUS in HF showed that higher number of B-lines was associated with higher risk of outcome irrespective of clinical settings (outpatient clinic RR: 1.66, 95% CI: 1.28-2.15; at admission RR: 2.32, 95% CI: 1.46-3.70; at discharge RR: 2.46, 95% CI: 1.56-3.86) and LUS-guided therapy in clinical trials was associated with reduced risk of adverse outcomes (0.50 (95% CI: 0.35-0.72) [66].

However, B-lines are not specific to HF and are seen in other causes of partial pulmonary deaeration such as pulmonary fibrosis, as well as acute lung injury, acute respiratory distress syndrome, and interstitial pneumonia. Additionally, absence of pulmonary edema does not exclude HF [62]. The number of B-lines may be influenced by BMI [71] or in cases of isolated right HF. Moreover, B-lines have been established as an independent prognostic marker but their prognostic utility of B-lines on top clinical risk scores has not been sufficiently established, and it is unclear if sequential assessment of B-lines changes can provide prognostic or therapeutic insights in HF. Additionally, how other clinical and imaging congestion markers influence the prognostic utility of LUS findings requires further studies. Therefore, B-lines findings should be interpreted cautiously in these circumstances and should be complemented with cardiac and venous ultrasound.

2.4.3.3. Point of care ultrasound and VExUS Score

Point of care ultrasound (POCUS) provides rapid and reliable assessment of venous congestion in multiple organs. In a point of care ultrasound, treating physician measures the IVC diameter, hepatic, portal and renal venous congestion using Doppler ultrasound. While conventional echocardiography provides detailed information on cardiac function, the advantage of POCUS is that it provides a rapid overview of vascular congestion in different organs. It is useful in bedside assessment of congestion and adapting treatment strategies.

Recently, Beaubien-Souligny et al., proposed a Venous Excess Ultrasound (VExUS) grading system to assess severity of venous congestion [72]. This score combines IVC diameter and venous Doppler

waveforms of the portal, hepatic and interlobular renal veins to assess the severity of congestion. A recent study reported that a VExUS score of 3 was associated with higher odds of in-hospital mortality (odds ratio, 8.03; 95% CI [2.25-28.61], P = .001) [73]. Further, VExUS score 3 was independently associated with were higher mean E/e' ratio, larger right ventricular size, severe tricuspid regurgitation, and impaired right atrial function in hospitalized patients. Similar, results were reported in ambulatory patients where higher VExUS score was associated with higher rates of the primary composite endpoint of all-cause mortality or HF hospitalization within 90 days (87% vs. 19.8%, $p < 0.001$), worsening renal function (47.8% vs. 17.3%, $p < 0.001$), and unplanned visits (82.6% vs. 14.8%, $p < 0.001$).

However, there are limited studies on clinical and prognostic utility of VExUS score in different HF populations. Implementing VExUS score would require standardization of protocols and training of physicians to obtain accurate and reproducible measurements [74].

2.4.4. Laboratory markers of congestion

2.4.4.1. Natriuretic peptides

BNP and its pre-hormone NTproBNP are the endocrine hormone secreted by ventricular myocytes in response to pressure or volume overload. NP plays an important role in counteracting RAAS overstimulation by exerting natriuretic and diuretic effects [75]. HF guidelines strongly recommend NTproBNP and BNP measurement in patients with suspected HF to rule out the diagnosis [5,75]. BNP values of more than 35pg/mL and that of NTproBNP more than 125 pg/mL indicate high likelihood of HF as the negative predictive value of values below these levels is shown to be in range of 0.94 to 0.98 [5,76,77] . BNP has a shorter half-life (20 mins) than NTproBNP (120 mins) and therefore BNP levels falls much faster than the NTproBNP levels. However, NP values may be elevated in old age, renal dysfunction and AF whereas, NP levels may be disproportionally low in cases of obese individuals [5].

Despite these pitfalls NP levels provide an easily measurable and repeatable, objective criteria for HF diagnosis and can identify population at higher risk of adverse events [75]. NP are one of the strongest single predictors of prognosis in HF [75]. In acute HF, patients who had lower NP levels or those who had more than 30% reduction NP levels had better prognosis than the patients who had higher levels at discharge[78]. Recent trials in HF included NTproBNP levels as eligibility criteria for patient inclusion. In both PARAGON-HF and EMEROR-Reduced trials patients with higher NTproBNP levels had higher risk of hospitalization for HF or cardiovascular deaths [79,80]. Moreover, NTproBNP have shown to be useful in identifying sub-groups of patients who may benefit from HF therapy. In VICTORIA trial, greater clinical benefit was observed with vericiguat in patients who had lower NP levels [81] whereas, in GALACTIC- HF trial, omecativ mecarbil was more beneficial in patients with low NP levels [82]. Serial NP levels have also been evaluated in clinical trials to test if NP guided therapy improves outcome compared to usual care. The results did not show statistically significant benefits in NP guided groups [83].

2.4.4.2. Estimated plasma volume status

Plasma volume expansion is associated with increased risk in HF. However, measuring exact plasma volume requires specialized equipment and may not be readily available in clinics/ hospitals. Change in hemoglobin or hemo-concentration can be used to estimate plasma volume reliably [84]. Various formulas such as Strauss, Hakim's and Duarte's have been used to estimate plasma volume. These formulas consist of change in hemoglobin levels or require hemoglobin and hematocrit levels. In the post hoc analysis of ESCAPE trial it was demonstrated that baseline to discharge increase in hematocrit, albumin or total protein values were associated with improved survival [85]. Similar findings were reported in post hoc analysis of GISSI-HF trial where increase in estimated plasma volume was associated with increased risk of ACM [86]. Moreover, estimated plasma volume has been shown to be associated with E/e', PCWP and natriuretic peptides in HF patients [84].

2.4.4.3. Other biomarkers: adrenomedullin and CA125

Adrenomedullin

Adrenomedullin is a small peptide hormone (52 amino acids) which is secreted by endothelial and vascular smooth muscle cells in response to volume overload [87]. It causes vasodilation and maintains vascular integrity and barrier permeability [9,87]. Higher levels of adrenomedullin are associated with severe HF and worst prognostic outcome [87]. In the RELAX-AHF-2 trial, pro-adrenomedullin was associated with higher congestion scores and in-hospital worsening. Further, adrenomedullin levels at discharge were associated with higher post-discharge mortality [88]. Recent studies show that biologically active ADM (bio-ADM) was associated with the presence of congestion signs such as oedema, orthopnoea, hepatomegaly, and elevated JVP [89,90]. Further, bio-ADM correlated with mean right atrial pressure whereas, NTproBNP correlated with pulmonary arterial wedge pressure indicating the bio-ADM as biomarker of venous congestion in a large community-based cohort (n = 5060) [91]. These studies indicate that bio-ADM reflects both tissue and vascular congestion. However, more research is needed if its level are influenced by age, BMI, hepatic and renal function and if it can be used for guiding decongestion therapy.

CA125

CA 125 is a glycoprotein that is widely used for monitoring ovarian cancers. It has been postulated that elevated hydrostatic pressure, mechanical and inflammatory stress are some of the causes that trigger CA 125 upregulation. The exact role of CA 125 is not exactly known however, several studies show that it is associated with severe congestion and worse outcomes in HF [9,92]. In a cohort of acute HF patients, it was observed that long-term trajectory of CA125 levels in combination with NTproBNP levels delineated risk of mortality. Patients in whom CA125 levels remained elevated after discharge were at increased risk of death [93]. Recent studies show that CA 125 is associated with high intra-renal venous pressure and intra-abdominal pressure [94,95]. CA-125 was also investigated as a biomarker for guiding

post-discharge diuretics and statin therapy in acute HF in Carbohydrate Antigen-125-guided Therapy in Heart Failure [CHANCE-HF] [96]. In this study, researchers titrated diuretics and statin dose, and increased monitoring with an aim to reduce CA-125 below 35 U/ml in CA-125 guided arm. It was found that CA-125 guided therapy was associated with reduced adverse outcome, mainly driven by reduced hospitalization for HF, compared to standard of care arm.

CA 125 may be a potential biomarker of predominantly systemic and tissue congestion. However, it is not a HF specific biomarkers and can be raised in several other conditions such as cancers and inflammation. Moreover, CA125 has along half-life (5-12 days) and may not reflect the acute congestion or rapid decongestion in acute HF [9]. Therefore, it is imperative that raised CA-125 levels be interpreted in conjunction with other more specific biomarkers of HF such as raised NP and echocardiographic parameters.

2.4.5. Forrester classification

Forrester classification is used to classify acute HF patients based on bedside evaluation of congestion and perfusion [97]. Although Forrester classification was initially described for classifying congestion and perfusion based on cardiac index measured by right heart catheterization in acute myocardial infarction patients. However, because of its invasive nature patients are now often classified based on clinical examination. Patients are classified as wet or dry depending on the presence of congestion and warm or cold if hypoperfusion is present or absent creating 4 distinct profiles: 'wet-warm' – patients having congestion and adequate peripheral perfusion; 'wet-cold' – with congestion and hypoperfusion; 'dry-cold' – free of congestion but with hypoperfusion; and 'dry-warm' – free of either congestion or hypoperfusion [97]. Different profiles identified through Forrester classification benefit from different therapeutic approaches and are associated with different long-term risk of outcome [5,97,98]. Patients with wet-cold profile may benefit from more aggressive therapy and invasive procedures. In the

EAHFE registry which included 11, 261 acute HF patients showed that hypoperfused patients were at higher risk of rehospitalization and in-hospital mortality whereas congested patients had prolonged hospital stay [99]. In ESC-EORP-HFA Heart Failure Long-Term, compared to dry-warm profile , wet-warm was associated with higher risk (HR: 1.46 (1.31–1.63)) and compared to wet-warm profile, wet-cold profile was associated with higher risk (HR: 2.20 (1.89–2.56)) [97]. **Importantly, the risk associated with higher number of B-lines was more compared to the risk associated with of wet-warm profile in HF patients (**at discharge RR: 2.46, 95% CI: 1.56-3.86**).** Distinguishing between systemic and pulmonary congestion within Forrester classification can improve risk stratification in advanced HF [100]. However, **the major limitation of Forrester classification is that it oversimplifies congestion as a single, binary state (only on presence or absence of congestion). It does not account for the distribution, extent, or severity of congestion across different clinical presentations.** This is a significant drawback because the degree and systemic involvement of congestion would likely permit an optimal clinical management. Further, this classification does not take into account several important prognostic markers such NP levels, renal function and other comorbidities.

2.4.6. Current approach to congestion management

Effective congestion management requires both accurate assessment and a multidisciplinary approach to have adequate long -term decongestion. The management plan vary depending on the clinical phase i.e., admission, discharge and post-discharge. In-hospital management focuses on identifying the congestion profile, degree of hemodynamic instability and possible trigger of acute decompensation. Clinical congestion scales, NTproBNP and bedside ultrasound can be used to guide therapy to achieve optimal decongestion [5,101]. Pre-discharge and long-term management includes planning the post-discharge visits and optimization of guideline directed medical therapy (GDMT). ESC-HF guidelines

recommends that a follow-up visit must be planned withing 1-2 weeks of discharge to reduce the risk of 30-day readmission [5].

Loop diuretics are the cornerstone of congestion management to achieve euvolemic status. Diuretics increase renal sodium water excretion thereby reducing vascular and extravascular fluid load. However, diuretic dosing can be challenging in presence of renal dysfunction and electrolyte imbalance. Diuretic dose intensification should be done in light of diuretic response and resistance. MRAs, acetazolamide, thiazide and thiazide-like diuretics can be added to achieve optimal decongestion. Further, up-titration of GDMT improves cardiac functioning which alleviates congestion symptoms. Non-pharmacological therapies such as salt-restriction, exercise rehabilitation, patient education and self-care are beneficial in long-term management of congestion. Remote monitoring and telemedicine may play an important role in identifying and managing congestion symptoms early which can prevent decompensation [101]. Integrating telemonitoring of signs and symptoms, assessment of biological markers at community health centers and treatment optimization plans according to congestion profile have the potential to improve patient outcomes.

Congestion is highly heterogenous; its distribution, pattern and severity depends on interaction of cardiovascular pathophysiology, comorbidities, response to treatment and individual susceptibility to pressure-volume overload. To exemplify, a patient having only systemic vascular congestion may follow different clinical trajectory and might benefit from adjusted treatment plan. Currently, congestion assessment is done using clinical examination, echocardiography and levels of NP biomarkers. **Despite congestion being the primary cause of hospitalization in HF, the existing congestion markers exhibit a moderate association with outcomes.** Importantly, available congestion markers rarely guide treatment decisions, most likely because our understanding of congestion remains too superficial. NP driven HF treatment did not improve patient outcomes in randomized clinical trials. **Currently, no**

precise or validated method exists which provides precise congestion segmentation needed to classify or identify congestion profiles. This gap probably exists because complex interactions of multiple congestion variables are not always discernible through conventional observation or conventional analytical approaches. Moreover, until recently, medical statistics often lacked the application of agnostic/ a priori-free approaches. **Several papers support the concept of classifying congestion to predominant phenotype to improve decongestion strategies [3,8]. However, these concepts are driven by expert opinion rather than emerging from a data-driven analytical process.**

2.5. Role of AI in identifying congestion phenotypes

The inherent heterogeneity of congestion coupled with complex underlying pathophysiology makes its classification challenging. Boorsma et al., and de la Espriella et al., provide a novel approach of congestion classification but this approach is largely eminence driven rather than data-driven [3,8]. This means that this approach of congestion classification relies on expert consensus and established clinical understanding and does not emerge from comprehensive data analysis.

Data-driven approach offers us a *tabula rasa* (clean-slate) which is free from prior assumptions and rules and pre-defined diagnostic criteria, thus minimizing human bias in analysis. Given the heterogeneity of congestion parameters, data-driven approaches which have the potential to consider multiple interlinked factors could be better suited to identify more uniform profiles than convention statistical methods. They may provide a better insight into the intricate relationships between various contributing factors.

The principal advantage of machine learning is that a large amount of data consisting of multiple variables can be analyzed, even if inherent correlations are present between the variables. In context of HF, machine learning techniques have been applied to improve diagnostic accuracy, predict survival and identify more homogenous HF profiles in patients with both acute and chronic HF [102-105]. Unsupervised machine learning models have been used to identify homogenous patient population within the heterogenous populations whereas, supervised machine learning models such as decision tree, random forest and extreme gradient boosting are used for prediction.

2.5.1. Unsupervised machine learning models

The unsupervised machine learning models that have been most commonly employed to identify HF phenotypes are K-means, hierarchical ascendent clustering, latent class analysis

and latent class model (LCM). The advantages and limitations of these methods are presented in table 1 below.

Table 1: comparison of clustering methods commonly used in medical research

Method	Key Advantages	Key Disadvantages/Limitations
K-Means	Conceptually simple and computationally very efficient for large datasets.	Requires pre-specified number of clusters beforehand, which is often arbitrary. Results can vary significantly based on initial centroid placement. Outliers can heavily influence cluster centroids. Only Numerical Data can be analyzed; requires transformations for categorical data.
Latent Class Analysis (LCA)	Probabilistic Assignment of class Well documented in clinical studies Identify patient subgroups from categorical data.	Requires choosing number of classes, which can be ambiguous. Continuous data needs to be converted which leads to loss of information; conversion is cut-off dependent
Latent Class Model (LCM)	Handles both continuous and categorical data directly. Probabilistic Classification.	Models are complex; computationally demanding Clinical meaning of classes still requires significant domain expertise.
K-Prototypes	Handles both numerical and categorical variables without transformation.	Requires choosing number of clusters without statistical fit tests. Results can vary based on starting points. No probabilistic membership. Outliers can heavily influence cluster centroids.
KAMILA (KAy-Means for MIxed LArge data sets)	Mixed Data Handling: Natively designed for mixed data types. Efficient for large datasets.	Newer Method; few studies have used this method.

The choice of model depends on size and type of data: K-means and hierarchical clustering are more suitable for continuous variables whereas, latent class analysis model requires data in categorical format. **LCM can analyze heterogenous data consisting of both categorical and continuous variables.** In a previous analysis conducted in our lab (CIC-P, Nancy) on a stimulated dataset, it was shown that the performance of LCA improved with increase in number in population whereas, Kamila, K-proto (to a lesser extent) and LCM

showed consistent performance regardless of sample size [106]. Also, LCM had good performance (high and stable ARI) when proportion of continuous variables were increased and at varying proportions of relevant variables (**Figure 5**)

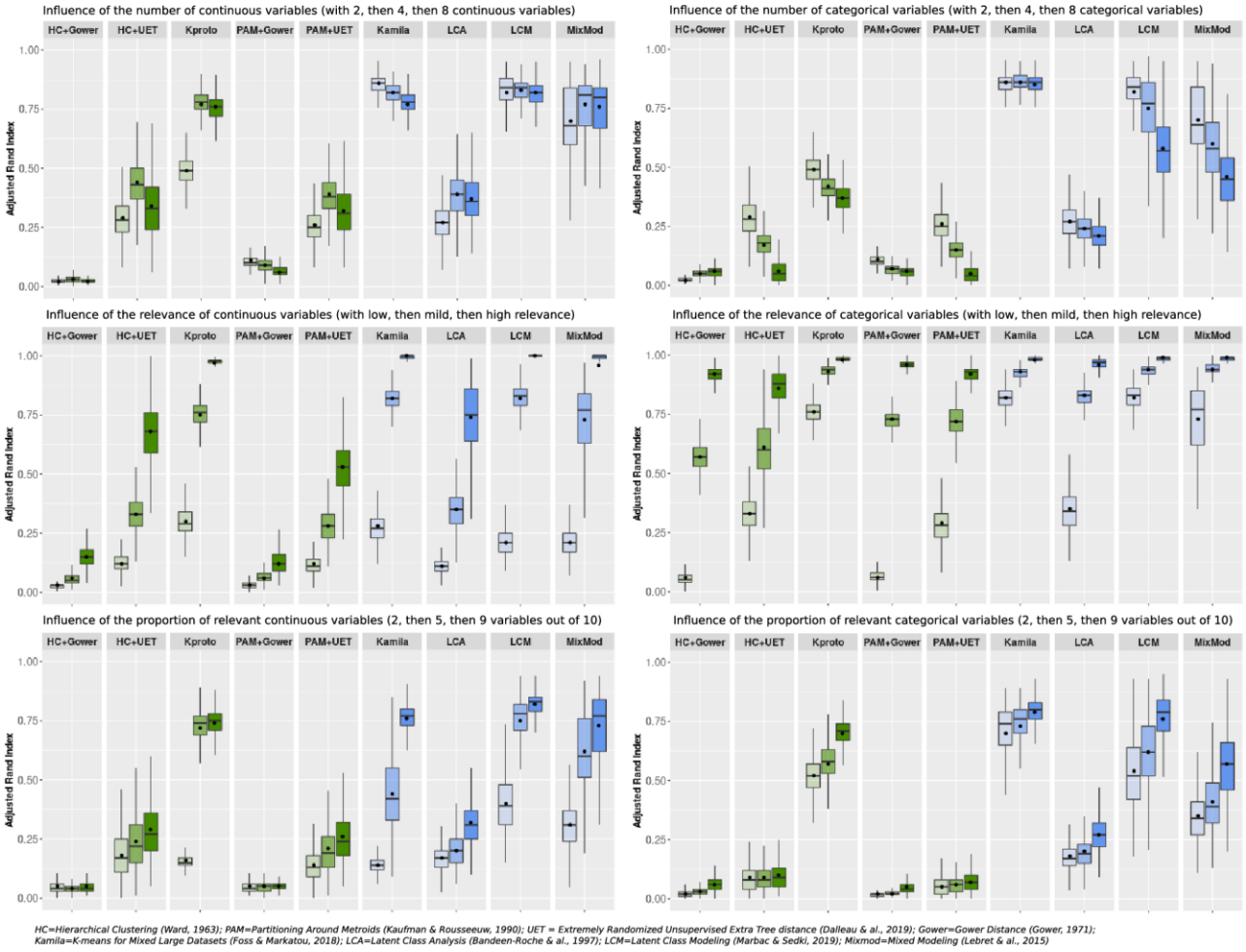

Figure 5: Influence of characteristics of continuous and categorical variables on clustering performance in simulation studies. (From Prud'homme et al., [106])

A head-to-head comparison of clustering methods by Prud'homme et al., [106] explored distance-based and model-based clustering methods in EPHESUS study dataset (**Figure 6 and 7**). The authors predefined number of clusters to 4 to study prognostic ability, predictive ability and characteristics of the cluster identified through different methods. The results demonstrated that LCM had good performance in identifying clinical profiles across clusters, prognostic performance and profiles with treatment benefits. More importantly, amongst both distance-based methods (hierarchical clustering, K prototype and partition around medoids)

and model-based (Kamila, latent class analysis, latent class model and mixmod), only LCM identified a cluster with important treatment benefits.

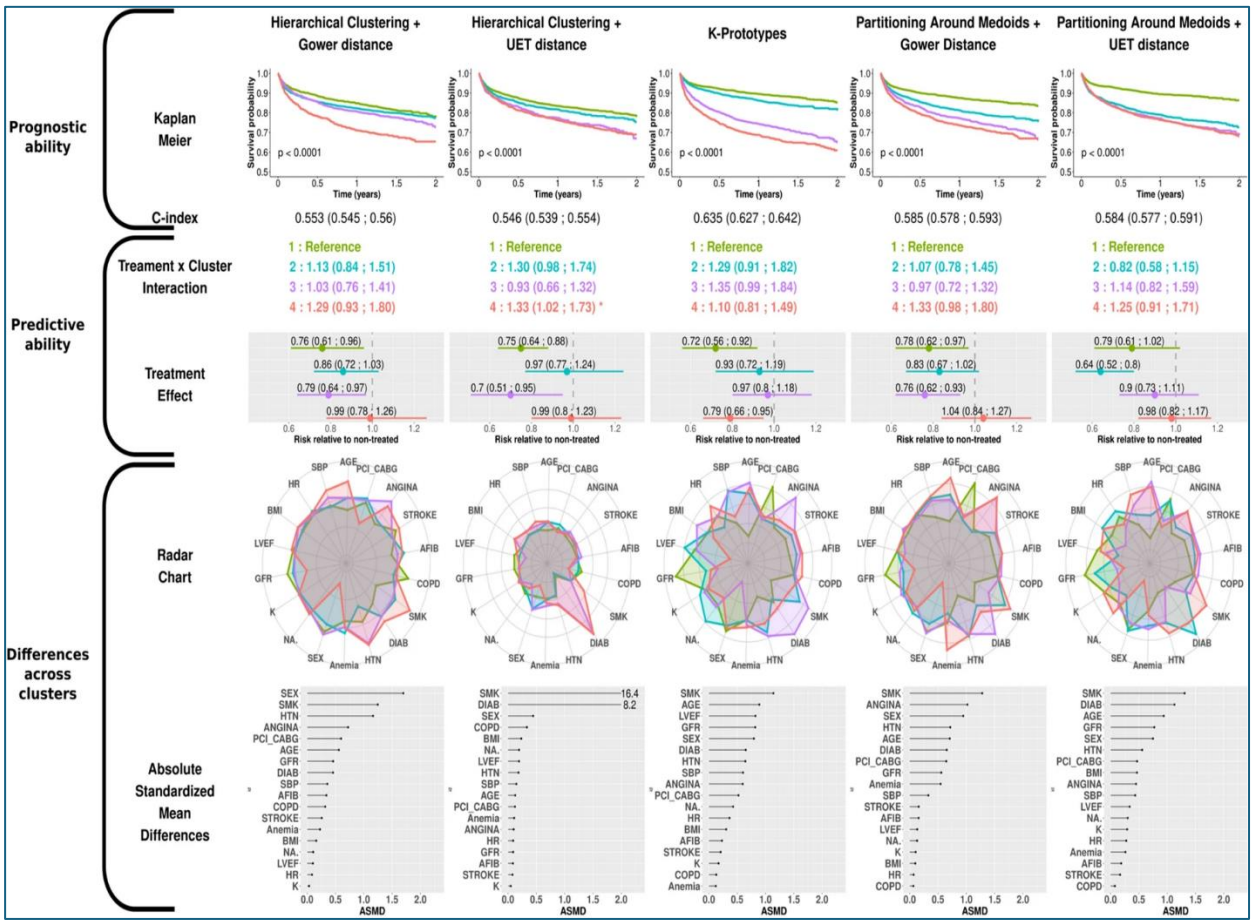

Figure 6: Results obtained with the distance-based clustering algorithms in the EPHESUS dataset. Radar charts were created from the means (per, by) group on scaled values (dichotomous and ordinal variables were coded numerically for convenience). The dot charts show the average Absolute Standardized Mean Difference (ASMD) over all clusters. (From: Prud'homme et al., [106])

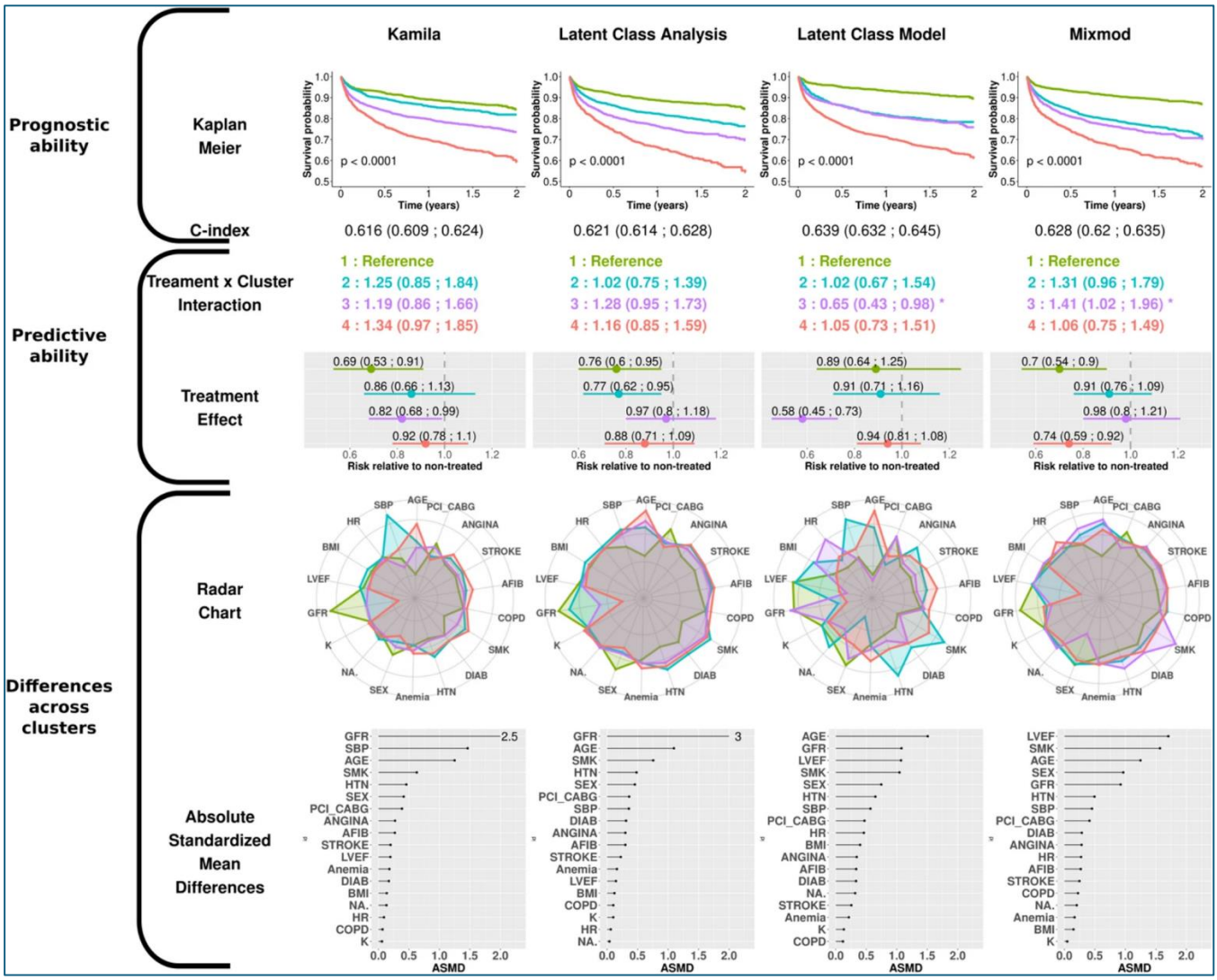

Figure 7: Results obtained with the model-based clustering algorithms in the EPHESUS dataset. Radar charts were created from the means (per, by) group on scaled values (dichotomous and ordinal variables were coded numerically for convenience). The dot charts show the average Absolute Standardized Mean Difference (ASMD) over all clusters. (From: Prud'homme et al., [106])

Identifying clusters and validating the developed AI model requires rigorous methodology to ensure clinical utility of the clusters [107]. Briefly, the first step of the process is cleaning and adapting data according to the chosen AI model. Once the clusters are identified they should be tested for internal as well as external validity. This process is delineated in figure **8** below:

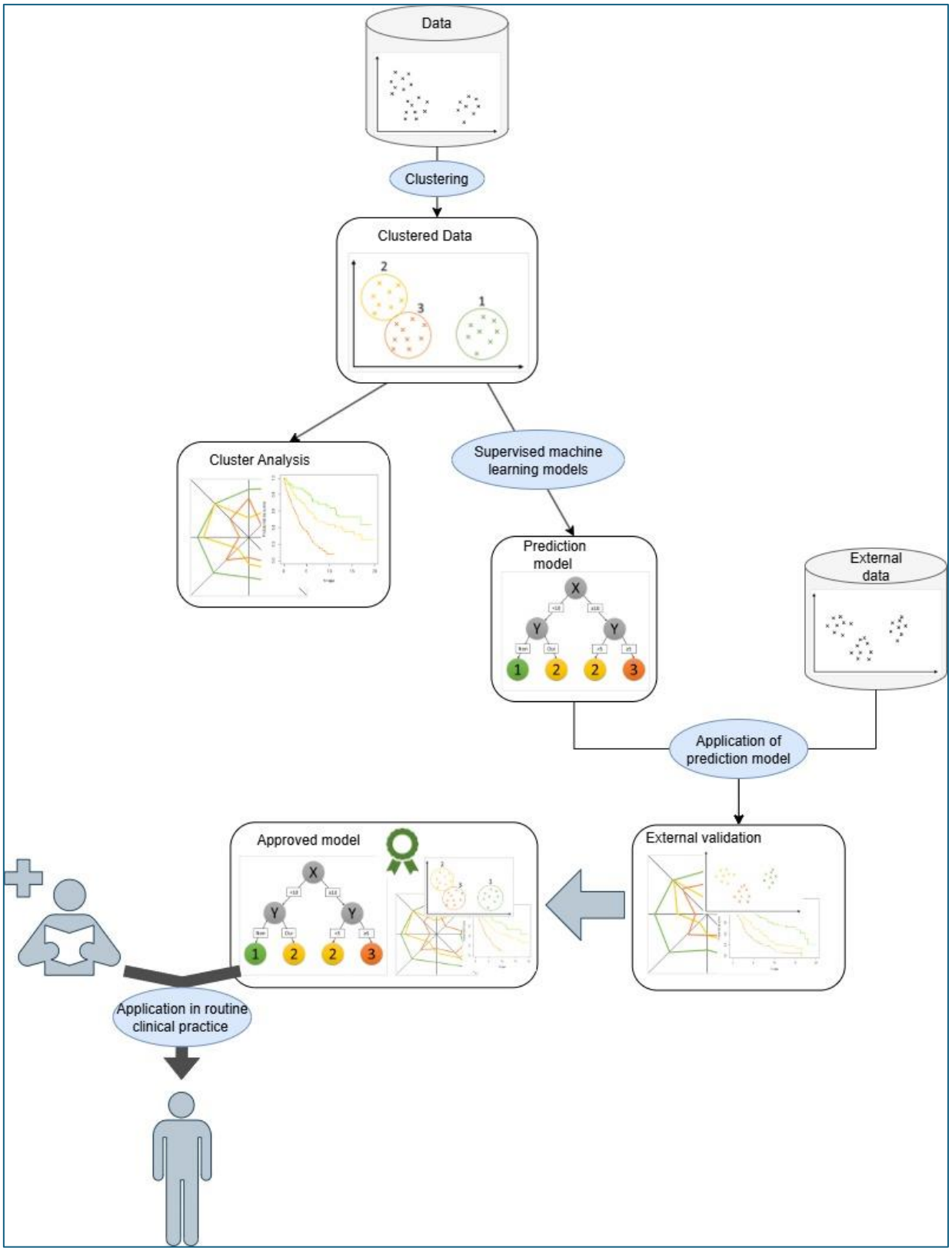

Figure 8: Process for clustering using machine learning models (adapted from Girerd et al.,[107]).

2.5.2. HF phenotypes identified by machine learning

Over the last decades, several studies have explored clustering methods to classify HF with an aim to improve its classification. Ahmed et al., published one of the first papers on classifying chronic HFrEF patients using hierarchical agglomerative clustering. In this analysis, clustering was performed on 45 clinical variables from ~1600 patients enrolled in HF-ACTION cohort. Clustering analysis identified 4 different clusters which differed in their demographic profile, presence of comorbidities, quality of life and biomarker levels. Importantly, these phenotypes had differential associations with outcome [108]. Similarly, Shah et al., conducted hierarchical clustering analysis using clinical, laboratory, ECG, and echocardiographic data in 397 HFpEF patients [105]. **Clustering analysis was able to identify meaningful profiles that differed in clinical characteristics and hemodynamics and prognosis in patients with HF**. One of the principal advantages of machine-learning based methods is that a large number of variables from different domains can be considered for analysis and model building. For instance, in a study in BIOSTAT-CHF cohort, 363 protein biomarkers were considered in the unsupervised clustering analysis to identify distinct pathophysiological profiles of HFpEF [109] whereas in another study 87 different variables from a wide range of domains such as demographic, comorbidity, blood tests and echocardiographic features were considered for latent class analysis [110]. In a study by Zhang et al., data from proteomics and metabolomics was integrated to study mechanisms in patients with HF and AF [111].

However, **very few studies have explored the congestion parameters to identify congestion phenotypes in HF**. Niimi et al., performed clustering analysis on signs of physical congestion (JVD, leg edema, S3, crackles, and orthopnea) in patients with acute HF [48]. Authors identified 3 phenogroups which were labelled as no physical congestion, congestion without JVD and congestion with JVD. Among these 3 clusters congestion with JVD had higher risk of outcomes (HR: 1.79, 95% CI 1.26–2.55) compared to patients with no physical congestion.

This study demonstrated that congestion signs and symptoms can adequately identify congestion profiles using clustering.

Though a large number of studies identified cluster phenotypes, only a few of them validated models in internal or external datasets thus limiting the generalizability of results. More importantly, the models developed to predict cluster class were not reported and hence, cannot be tested with independent/external cohorts. Moreover, most of the studies conducted clustering analysis on limited variables from demographic, comorbidity profile, laboratory and/ or imaging domain. Further, heterogeneity in clustering approaches, variable selection and criteria for number of cluster selection makes it difficult to compare the results from different studies. In addition, several studies did not report details on variable selection, data imputation and methodology [112]. A guiding document/consensus which can harmonize methodological aspects of reporting clustering driven profile is needed.

Machine learning driven models have been explored for range of applications in HF such as diagnosis, predicting response to therapy, predicting adverse outcomes and phenotyping. However, **a major deterrent to their integration in clinical practice is the lack of standardization in developing and reporting the algorithm as well as scarcity of validation in diverse populations.** More studies where AI models are validated and shared for further validation are therefore required. As AI driven research in health sciences is in their nascent stages, most of the studies have used known established risk factors or prognostic markers to explore HF phenotypes. **An approach which considers wide spectrum of congestion markers may considerably broaden our understanding of HF phenotypes. This is particularly important, as HF pathophysiology is not yet well understood, and involves several gene-protein-metabolite interactions that influence the regulatory and counter-regulatory mechanisms.** Unlike, hypothesis driven approach where a few biomarkers are

assessed for their pathophysiological role in disease, AI driven models have the capacity to concomitantly analyze a wide array of biological data. This could unveil pathways and biomarker interaction involved in congestion profiles, possibly informing on disease progression, patterns and therapeutic response.

2.6. Biological background of congestion

2.6.1. Renin - angiotensin - aldosterone system

The role of renin - angiotensin - aldosterone system (RAAS) has been widely studied in context of HF. RAAS axis works in both paracrine and systemic endocrine levels [113,114]. RAAS plays an important regulatory role in maintaining sodium water balance and aldosterone secretion. It regulates tissue perfusion by regulating cardiac function and vascular tone. Renin, initiator of RAAS cascade, is secreted from juxtaglomerular cells in response to change in renal perfusion and/ or beta-1 receptor activation [113,114]. Renin converts angiotensinogen to angiotensin-I, which is converted to Angiotensin-II by angiotensin converting enzyme (ACE). Angiotensin-II, the primary effector molecule of RAAS system, acts on angiotensin receptors. Angiotensin-II regulates renal hemodynamics by constricting efferent arterioles to increase sodium and water resorption. Further, it increases aldosterone secretion from adrenal cortex. Aldosterone increases sodium reabsorption from renal cortical collecting duct.

Initially, RAAS activation is a protective compensatory mechanism in HF triggered due to “arterial underfilling” and renal hypoxia. However, **prolonged activation of RAAS has deleterious effects on cardiovascular and renal system. It is associated with salt/water retention, hypertension, vascular smooth muscle hypertrophy, cardiac remodeling and renal fibrosis.**

Drugs that inhibit RAAS system such as ACE inhibitors, angiotensin receptor blockers and mineralocorticoid antagonists are the cornerstone of HFrEF therapy. These drugs reduce cardiac remodeling and improve prognosis in HFrEF. These medications are recommended as Class IA in the 2021 ESC guidelines [5]. RAAS inhibitors are not effective in reducing mortality and morbidity in HFpEF, however candesartan and spironolactone reduce hospitalization for HF [5]. Recent studies suggest machine learning models can be used to predict the risk of hyperkalemia and renal adverse events associated with RAAS inhibitor [115] and identify phenotypes having potentially different response to eplerenone [116].

2.6.2. Fibrosis

Cardiac fibrosis is an evolutionary process which seems to be in response to injury, inflammation or stress resulting from ageing, cardiometabolic diseases such as hypertension, diabetes, coronary artery diseases [117]. Various mechanisms that lead to cardiac fibrosis have been proposed over the years. Comorbidities such as diabetes, CKD and obesity have been associated with high levels of inflammatory and fibrotic markers like GDF-15, interleukins and CRP. Analysis of circulating proteins in BIOSTAT-CHF and PROMIS HF have shown that pathways related to inflammation and extracellular matrix (ECM) organization were overrepresented in patients with HF. In addition, hypertension is associated with increased proinflammatory[118,119] and profibrotic signaling [120,121]. Animal studies have demonstrated that pressure overloaded myocardial cells produce cytokines and chemokines leading to macrophage and inflammatory cell recruitment. These changes could facilitate procollagen processing and collagen fiber assembly and further fibroblast activation and deposition of collagen proteins.

Myocardial biopsy of HF patients with hypertension and AF show increased deposits of collagen in the ECM. Further, MMP resistant collagen with higher cross-linking was associated with worse prognosis in HF [122]. In addition, metabolic and hemodynamic load may also lead to shift towards collagen type 1 from collagen type III [123]. Collagen type I is less compliant compared to Type III collagen. The accumulation of collagen fibers leads to alteration of ECM and myocardial stiffness. Further, ECM deposition is linked to structural deformity, arrhythmia and diastolic dysfunction [124].

While the endomyocardial biopsy is the gold standard for diagnosing myocardial fibrosis, its invasive nature limits its use in regular clinical practice. **Assessment of circulating biomarkers of proteins is useful indirect measures of myocardial fibrosis.** Several studies have shown that biomarkers of collagen synthesis and degradation correlate with

histologically confirmed myocardial fibrosis severity. Most commonly studied biomarkers are PICP, PIIINP, PINP, CITP and CITP/MMP ratio [125]. These markers have been studied as markers of response of anti-fibrotic therapies.

While there is concrete evidence of cardiac fibrosis in HF, the mechanisms or biological pathways are not delineated. A recent study to understand the pathophysiology of congestion in BIOSTAT-CHF showed that patients with congestion had higher levels of FGF-23, FGF-21, CA-125, soluble ST2, GDF-15, FABP4, IL-6, and BNP [92]. Further network analysis in the aforementioned study indicated a cross-talk between endothelial dysfunction, inflammation and extracellular matrix deposition. In addition, in a post-hoc analysis of HOMAGE, higher LAVi was associated with several proteins of fibrotic pathways such as NT-proBNP, MMP-2, and IGFBP-2 [126]. In patients with acute HF, hemodynamic congestion measured with peripheral venous pressure correlated with collagen type IV N-terminal, a marker of liver fibrosis. Further, sustained elevation of peripheral venous pressure was associated with persistent increase in circulating collagen type IV N-terminal protein.[127] **However, how fibrotic markers impact congestion onset or progression or vice versa is not yet well understood**.

2.6.3. Holistic approaches

Conventionally, medical science has approached any given disease with a reductionist viewpoint, that is, to explain the disease in a simplified manner by breaking down the pathology to a change in a single protein, gene alteration or functional dysregulation [128]. For example, it is well evident that **HF is a heterogenous complex syndrome, yet it is classified and treated based on LVEF. This segmentation does not encompass the demographic, genetic or comorbidity risk profile of patients** even when it is known that there sex differences in response to treatment response and distinct phenotypes exist within HFrEF population [129,130].

2.6.3.1. Multi-omics and proteomic based approaches

Emerging technologies in image acquisition, biological data assessment, such as multi-omics, and advanced statistical analysis is creating a paradigm shift in the medical sciences towards holistic approaches. Among these techniques, multi-omics approaches have recently gained popularity. Multi-omics approach includes study of genetic mutations and polymorphisms (genomics), gene expression (transcriptomics), small molecules (metabolomics), proteins (proteomics), immunophenotyping, and lipids (lipidomics) in biological fluids or tissues. Genomic analysis has been used for discovering genetic variants associated with HF onset and develop polygenic risk scores to account for genetic risk whereas, proteomic analysis had been used to identify HF subtypes and possible drug targets [131]. A more holistic understanding emerges from sequential and layered multi-omics data analysis. This approach integrates different datasets to identify novel biomarkers for early detection and risk prognosis, as well as new treatment targets. Sequential proteomic and metabolomic profiling in Jackson heart study showed that leukotriene A4 hydrolase (LTA4H) was strongly associated with LV hypertrophy in Black population [132]. Further, this association was reflected in the downstream metabolites of leukotrienes and LV mass. Corroboration from multi-omics approach increases the validity of study results and identifies strongest possible candidate which can be analyzed as biomarker or treatment target. **Omics- based approach would be crucial to develop targeted therapies in line with the molecular underpinnings of the disease and identifying common targets for multiple HF phenotypes**.

Circulating levels of natriuretic peptides, adrenomedullin, and other specific proteins provide important information on myocardial stretching and vascular leakage yet, these proteins do not reveal the wide-spectrum of other possible pathways mediating congestion related organ damages such as those involving hemostasis, renal injury, inflammation and fibrosis. **Proteomic approaches study a wide spectrum of proteins expressed by cell, organs or a system.** Presently, four methods of high throughput screening are commonly employed to

identify and quantify the proteome: mass spectrometry, protein microarray chips, aptamers and proximity extension assay.

Proximity extension analysis is highly sensitive and allows for absolute quantification of a panel of proteins using extremely small samples (1 µL). In this method, oligonucleotide–labelled antibody probes bind to specific proteins targets [133,134]. Oligonucleotide pairs on the targeted protein hybridize to create a DNA amplicon with each having ID barcoding. The amplicons are subsequently quantified using real-time PCR. The platform can provide relative quantification of protein as $\log_2$ normalized protein expression (NPX) or absolute values depending on the panel used.

Proteomics based approach provides a unique benefit in understanding multi-organ impact as proteins are the effector molecules of gene expression and mRNA transcripts. **Protein expression is highly dynamic and changes in response to changes in environment, drug exposure and disease progression. Therefore, protein measures can also provide important clues on response to therapy to validate the role of protein and drugs in disease pathway.** Moreover, protein-protein interactions can provide important insights into the pathophysiological pathways and their regulations. Discovering important proteins linked to the pathophysiological modulations may prove to be useful in identifying diagnostic and prognostic biomarkers, biomarkers of different stages of HF and easily druggable targets [135]. In a population-based cohort (n= 768), Egerstedt et al., studied the proteomic profiles (1305 proteins) of prospective HF and patients undergoing heart transplant [136]. It was found that immunological, complement, coagulation, natriuretic and matrix remodeling pathways were modulated almost 2 decades before the incidence of HF (n = 185) [136]. In patients who underwent cardiac transplant, 138 of 421 proteins associated with incident HF regressed towards levels observed in heathy population after 6 months of transplant. These findings indicated towards the proteins that were deranged due to failing heart rather than due to comorbidities. Thus, providing insights into possible mechanisms in early and late HF. Similarly, proteomic analysis

provided insights into the biological mechanisms differing in patients with HFrEF vs HFpEF and in patients with HF and AF or in sinus rhythm [135].

2.6.3.2. Complex networks

Complex networks provide a framework to explore relationship between constituents of biological system at the same level and across different levels (such as cell, organ, individual and environment). Networks consists of interacting elements wherein each element is represented as a node and the interaction between two nodes as link [137]. This representation of networks is based on the "graph theory" from mathematics in which collection of all the nodes and links is called a graph. The graphs can be further detailed by showing directionality, weight or temporality. Several bioinformatic tools such as Reactome, Gene Ontology, Wiki pathways and Kyoto Encyclopedia of Genes and Genomes (KEGG) are used to understand the functions, relations and biological processes of genes and proteins.

Application of complex networks in congestion/heart failure

Complex networks have been investigated to identify underlying protein-biological pathways in both healthy individuals and HF patients. In MEDIA-DHF cohort, authors explored the mechanism underlying the sex differences observed in HF [138]. Of the 415 proteins analyzed, 9 proteins were differentially expressed between male and female patients. Network analysis revealed that the proteins which were upregulated in female patients were related to lipid metabolism, transcriptional regulation, and hemostasis whereas, proteins upregulated in male patients were linked to extracellular matrix organization and developmental processes. In HOMAGE and STANISLAS cohort, a complex network analysis to identify pathways linked to LAVi showed that extracellular matrix remodeling and collagen synthesis/degradation were the over-represented pathways [139]. These findings suggested the central role of fibrosis in HF. Similar findings were reported in a study conducted in COMMANDER-HF trial to explore

protein pathways in patients with HFrEF with CAD. It was found that the overrepresented pathways were associated with inflammation and remodeling mechanistic pathways [10].

2.6.3.3. Holistic approaches

To fully appreciate the multi-dimensional aspects of a disease both clinical phenotypes and molecular markers need to be seen side by side. A holistic approach integrates clinical and imaging data with "new" biology data (genomic, transcriptomic, proteomic, metabolomic) and non-traditional data sources (online database) to better explain complex biological systems involved in health and disease [137,140]. It is being increasingly accepted that **to understand many system-wide phenomenon a system needs to be studied as whole and not aggregation of its parts.**

Holistic approach could bring a paradigm shift in our understanding of health and disease. They can improve diagnostic and therapeutic accuracy by integrating data from diverse domains. For example, disease associated pathways can be identified by examining the connectivity between nodes and groups of nodes within those pathways.

However, there are several challenges in implementing holistic approach. Its application requires accurate assessment of clinical and subclinical disease along with detailed "omics" and imaging data. In addition, directionality of pathway perturbation is not always clear; it is often difficult to determine whether the perturbation in the node is due to the disease, a medication or if the protein perturbation itself is causing the disease. Moreover, these methods require storing and sharing large amount of data which are costly and can raise privacy concerns.

While RAAS system and fibrotic pathways partially explain the pathophysiological pathways involved in congestion onset and progression. Several other pathways related to oxidative stress and inflammation are worth exploring. **A number of congestion biomarkers have been explored individually yet a holistic approach to evaluating role of biomarkers in distinct congestion phenotypes is missing.** Integrating personalized medicine with

evidence-based medicine is a daunting task and till date, holistic approaches have not led to new therapies. It is to an extent due to scarcity of cohorts with well characterized clinical profiles and detailed multi-omics data. Moreover, such approaches require not only computational expertise but also cross-functional team.

2.6.3.4. Relevance of -omics based approaches in congestion classification

Pathophysiology of HF subgroups/phenogroups/ comorbidity profiles have been explored using multi-omics approach. However, till now very few studies have explored possible biological pathways using proteomic or multi-omics approach in context of congestion in HF despite the fact that congestion is closely related with worsening HF and prognosis. **Omics based approach could be useful in identifying which proteins are modulated in patients with congestion compared to dry patients.** Furthermore, proteomic based approach may identify diagnostic and prognostic biomarkers in patients with different severity of congestion. To understand pathophysiology, it may be important to identify proteins that are associated with decongestion. Moreover, proteomic analysis could provide answers to why some patient are resistant to decongestion or develop side effects. Recently, a study in Mechanisms of Diuretic Resistance Cohort examined 369 serum proteins to study their association with HF rehospitalization, mortality and diuretic response in patients with ADHF [141]. The study showed that CDH1, FABP6 and TNC were associated with mortality and MMP7, PGLYRP1 and REN proteins were associated with reduced diuretic response in ADHF. This study highlights that protein analysis has the potential to identify proteins with prognostic and therapeutic utility.

Congestion in HF is driven by crosstalk between dynamic cardiac function, mechanical stretch, cardiac structure, fibrosis, neurohormonal activation, inflammation, oxidative stress and renal function, etc. Yet, our understanding of biological mechanisms of congestion remains limited which hinders development of novel prognostic biomarkers and therapeutic targets. **Presently, congestion management is mostly symptomatic and tailored to hemodynamic**

status of the patients but does not take into account the underlying pathophysiology. The treatment algorithm of congestion is centered around decongestive drugs such as diuretics, MRAs and RAAS inhibitors which reduce fluid overload and neurohormonal signaling. In addition, MRAs limit profibrotic/inflammatory signaling and protects against cardiac remodeling. Current research suggests that SGLT2i have a pleiotropic effect in HF including, improving cardiac metabolism, natriuresis and reducing oxidative stress and fibrosis. Large body of evidence indicates that response to SGLT2in can be explained by increased autophagic flux (producing simultaneous upregulation of nutrient deprivation signaling and downregulation of nutrient surplus signaling) [142]. **Although inflammation, oxidative stress and fibrosis have an important role in worsening HF and congestion, drugs specifically targeting these pathways are not available.** Given the interconnectedness in the aforementioned congestion mechanisms, holistic approaches may be a way forward towards understanding congestion comprehensively. **Future research must analyze different domains of congestion, including cardiac structure and function, comorbidities, and genetic and protein pathways, to achieve a comprehensive understanding**.

In a nutshell, congestion in HF remains challenging to classify and characterize. We lack the robust evidence of prognostic utility of 1) quantifying pulmonary congestion assessed by imaging in patients with different phenotypes and clinical settings; 2) assessing changes in congestion through multi-modal assessment 3) combined assessment pulmonary and systemic congestion to congestion profiles. Furthermore, we have not yet explored congestion through the prism of agnostic approaches to understand underlying pathways of congestion profile.

In this research project, we investigate the prognostic utility of pulmonary tissue congestion assessed by LUS in patients with distinct clinical profile and settings, changes in several dimensions of congestion and combined congestion assessment through LUS and IVC ultrasound. We will employ clustering approach to identify congestion profiles and explore biological pathways through network analysis. Further, we develop a prediction model that can be easily used to identify a congestion class and validate it in external cohort.

3. Hypotheses and objectives

Congestion significantly impacts both HF symptoms and progression. A deeper understanding of its patterns and distribution can be beneficial in categorizing HF patients as well as developing personalized management strategies. Congestion can be broadly classified as intravascular and tissue congestion which can be further sub-divided into predominant pulmonary and systemic congestion. **While clinical congestion offers significant insights into differences in patients' characteristics and prognosis and is easy to implement, its reach is insufficient to fully understand congestion.** Integrating clinical, biological and imaging variables of congestion may improve HF categorization. Furthermore, machine learning approaches offer the potential to identify homogeneous congestion profiles by analyzing a wide array of variables. These data-driven, agnostic methods may either validate or challenge traditional conceptual frameworks that distinguish congestion based on systemic vs pulmonary and tissue vs hemodynamic components.

Hypothesis

We hypothesized that:

- Integrating clinical, biological and echocardiographic congestion data can better categorize HF.
- Assessment of bedside pulmonary congestion through LUS can provide better risk stratification in patients with HF regardless of clinical profile of patients.
- Quantum of in-hospital congestion changes, as well as combined pulmonary and systemic congestion assessment, can more accurately characterize patients at higher risk.
- Unsupervised machine-learning approach can identify, within the inherently heterogeneous population of HF patients, clinically meaningful informative congestion profiles.

- Distinct congestion profiles are linked to different risk of outcome, distinct biological signatures, different pathophysiological mechanisms and are possibly sensitive to molecules with different mechanisms of action.

Objectives

1. To assess the prognostic value of
    a. pulmonary congestion evaluated by B-lines across clinical profiles and clinical settings,
    b. in-hospital changes in congestion either evaluated by clinical, imaging or natriuretic peptide
    c. combined assessment of pulmonary (B-lines) and systemic congestion (IVC diameter) in HF.
2. To identify and characterize distinct congestion profiles in HF using unsupervised machine learning approaches integrating clinical, biological, and echocardiographic congestion variables and to assess their prognostic significance and underlying biological pathways through complex network analysis using proteomic data.

4. Introduction to Articles in thesis

During this thesis, I first-authored three articles (two published, one under review) and second-authored one published paper.

The first paper of this research (published) focused on the assessment of prognostic impact of pulmonary congestion (measured using LUS) in HF [143]. We assessed the prognostic relevance of B-lines in HF in different clinical settings (at admission, discharge and out-patient clinics) and according to different clinical phenotypes (age, sex, LVEF and presence/absence of AF) in a pooled data from international cohort. We found that a higher number of B-lines was associated with increased risk of outcomes at discharge and in out-patients but not at admission. We observed that prognostic capacity of B-lines was not influenced by age, sex, LVEF or presence of AF in the outpatient cohort. However, it was influenced by underlying heart rhythm (p-interaction = 0.0016) in the admission cohort and by sex (p-interaction = 0.0007) in the discharge cohort.

In the second (published) [144] and third (submitted, under review) paper, we investigated the prognostic relevance of combined assessment of pulmonary and systemic congestion. In the second paper, we focused on the changes in the levels of in-hospital congestion based on clinical signs, NP levels and pulmonary congestion [144] . We found that compared to patients with improvement in congestion levels, no/limited improvement in clinical congestion score or NP levels or B-line numbers was associated with poorer outcomes regardless of LVEF category. The event rates of outcomes varied for different combinations of improvement in congestion markers. Specifically, limited improvement in clinical congestion score and B-lines was associated with higher event rates in acute HF. Thereafter, in the third paper, we assessed the prognostic significance of the interplay between pulmonary and systemic congestion in HF using the pooled cohort data. We assessed how the coexistence of pulmonary and systemic congestion is associated with prognosis in patients with HF. We found that higher risk was

observed among in-patients and out-patients when systemic congestion was concurrent with more than mild pulmonary congestion. Our findings highlighted the improved prognostic capacity of integrated assessment of pulmonary and systemic congestion.

In the last part of our research, we focused on identifying congestion profiles using clustering analysis. We explored the underlying pathophysiology of congestion phenotypes based on systemic and pulmonary congestion parameters of the identified profiles. Clustering analysis could identify clinical congestion profiles associated with both long-term clinical risk and differences in biomarkers, suggesting potential different underlying pathophysiology. Network analysis conducted on circulating protein data identified distinct pathways associated with each congestion profile.

4.1. Prognostic value of pulmonary congestion assessed using LUS

4.1.1. Prognostic implication of lung ultrasound in heart failure: a pooled analysis of international cohorts

LUS provides a semi-quantitative measure of pulmonary tissue congestion. Bedside 8-zone LUS provides quick, accurate and reproducible quantification of pulmonary congestion. **However, whether clinical factors like age, sex and LVEF affect prognostic interpretation of LUS is not well known. Further, it was not well-established that if incorporating LUS findings in clinical risk scores improve risk stratification in HF.** Previous studies included patients in specific clinical settings, that is, either admission, discharge or out-patient clinics. Moreover, most of the previous study that assessed prognostic utility of LUS had a modest sample size which limited their generalizability; the largest sample size for the study in discharge patients was 132 and for the outpatients was 577 [145]. In this study, we assessed the prognostic relevance of B-lines in a broad-spectrum of patients with HF (n = 1947) in different clinical settings (at admission, discharge and out-patient clinics) and according to different

clinical phenotypes (age, sex, LVEF and presence/absence of AF). Further, we assessed the incremental prognostic value of LUS on top of key biological markers (natriuretic peptides and eGFR) and two validated clinical risk scores (AHEAD and MAGGIC) in patients with HF.

We pooled data from 8 international cohorts of patients with acute and chronic HF. In this study, patients were grouped into three sub-cohorts of admission (n=578), discharge (n=389) and in out-patient clinic (n=980). Eight-zone LUS protocol was used to calculate total number of B-lines. The primary outcome was a composite of re-hospitalization for HF and all-cause death.

We found that a higher number of B-lines was associated with increased risk of primary outcome at discharge (Tertile3 vs Tertile1: adjusted HR= 5.74 (3.26- 10.12), $p<0.0001$) and in out-patients (Tertile3 vs Tertile1: adjusted HR= 2.66 (1.08- 6.54), $p=0.033$). Adding B-line count on top of clinical AHEAD and MAGGIC risk scores improved the net reclassification significantly in all three clinical settings.

Our findings suggest that assessment of pulmonary congestion can provide better risk stratification regardless of clinical settings.

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ORIGINAL PAPER

Prognostic implication of lung ultrasound in heart failure: a pooled analysis of international cohorts

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Abstract

Background: Lung ultrasound (LUS) is often used to assess congestion in heart failure (HF). In this study, we assessed the prognostic role of LUS in HF patients at admission and hospital discharge, and in an out-patient setting and explored whether clinical factors (age, sex, left ventricular ejection fraction (LVEF) and atrial fibrillation) impact the prognostic value of LUS findings. Further, we assessed the incremental prognostic value of LUS on top of AHEAD and MAGGIC clinical risk scores.

Methods and Results: We pooled data of patients hospitalized for HF or followed-up in out-patient clinics from international cohorts. We enrolled 1,947 patients, at admission (n=578), discharge (n=389) and in out-patient clinic (n=980). Total LUS B-line count was calculated for the 8-zone scanning protocol. The primary outcome was a composite of re-hospitalization for HF and all-cause death. Compared to those in the lower tertiles of B-lines, patients in the highest tertile were older,

more likely to have signs of HF and higher NT-proBNP levels. A higher number of B-lines was associated with increased risk of primary outcome at discharge (Tertile3 vs Tertile1: adjustedHR= 5.74 (3.26- 10.12), p<0.0001) and in out-patients (Tertile3 vs Tertile1: adjustedHR= 2.66 (1.08- 6.54), p=0.033). Age and LVEF did not influence the prognostic capacity of LUS in different clinical settings. Adding B-line count to MAGGIC and AHEAD scores improved net reclassification significantly in all three clinical settings.

Conclusion: A higher number of B-lines in patients with HF was associated with increased risk of morbidity and mortality, regardless of the clinical setting.

Keywords: Cardiovascular diseases, Heart failure, Lung ultrasound, Cardiac oedema, B-lines, Prognosis

Introduction

Congestion is a key pathophysiological feature of heart failure (HF), and one of the main drivers of symptoms, signs, adverse remodelling and, eventually, hospitalization and premature death. (1-6) Accurate assessment of congestion can provide valuable information on the risk of adverse outcome and guide HF management.

Assessment of B-lines on lung ultrasound (LUS) emerged as a semi-quantitative, quick and reliable imaging tool to facilitate a diagnosis and quantification of pulmonary congestion in patients with acute or chronic HF (7) and improve risk stratification, as recently highlighted by an EACVI consensus document (8). Serial LUS can also be used for bedside adjustment of diuretics dose in HF. Another advantage of LUS is that it is useful in diagnosing HF correctly in primary care setting where natriuretic peptide may not be immediately available (9). Of the different LUS protocols proposed and used (4, 8, 28-zones), the 8-zone protocol combines good accuracy and reproducibility with shorter time for image acquisition and it might, therefore, be preferred to others (10). However, which clinical factors like age, sex or left ventricular ejection fraction (LVEF) may affect prognostic interpretation of LUS in patients with HF has not been well studied. Similarly, it is not known if LUS clearly improves risk stratification, mostly because of the small sample size of studies conducted so far (11, 12). Moreover, previous studies were focused either on the acute settings or the out-patients setting to assess the prognostic capacity of B-lines. However, in this paper, we take an integrative approach of assessing prognostic capacity of B-lines in different clinical setting in a pooled cohort of more than 1900 patients with HF.

Hence, in this research collaboration, we aimed to assess the prognostic relevance of B-lines in a broad-spectrum of patients with HF in different clinical settings (at admission, discharge and out-patient clinics) and according to different clinical phenotypes (age, sex, LVEF and presence/absence of atrial fibrillation (AF)). Further, we assessed the incremental prognostic value of LUS on top of key biological markers (natriuretic peptides and eGFR) and two validated clinical risk scores in patients with HF, AHEAD and MAGGIC.

Method

Patient population

This retrospective study is the collaboration between eight cardiology centres wherein we pooled the patient-level data from eight cohort studies: (***Supplementary table*** 1).

i) **The Nancy cohort study (admission):** Included patients hospitalised for acute HF at "Département de Cardiologie Medicale", Nancy, France from April 3, 2014 to August 4, 2015 (13). LUS was performed once, within 3 days of admission.

ii) **The AHF-core study (admission and discharge):** An ongoing cohort study (NCT03327532) which includes patients hospitalised for acute HF in the two university hospitals of Grand-Est region of France. LUS was conducted within 72 hrs of admission and again before discharge.

iii) **The Pisa cohort study (admission and discharge):** Included patients hospitalized for acute HF in the Cardiology Department in Pisa from October 2004 to October 2008 (3, 14). LUS was conducted within 24 hours of admission and before discharge.

iv) **The Siena cohort study (admission and discharge):** Included patients with new-onset or worsening HF in sinus rhythm admitted to the Cardiovascular Diseases Unit in Siena (15). LUS was performed within 12 hours of admission and before discharge.

v) **The Perugia cohort study (discharge):** Included patients hospitalised for acute HF at the Division of Cardiologia e Fisiopatologia Cardiovascolare in Perugia from April 2014 to October 2014 (16). LUS was conducted at discharge.

vi) **Nis cohort (out-patients):** The out-patient cohort enrolled patients with HFpEF referred to Cardiology Clinic of the Niska Banja Institut, Nis University, Serbia from June 1, 2016 to February 1, 2018 (17).

vii) **The Hull cohort study (out-patient):** Included patients with HF attending a specialist clinic for a routine follow-up visit from April 2016 to March 2017 (18).

viii) **The Barcelona cohort study (out-patient):** Included patients with HF attending a routine clinical appointment from 6 July 2016 to 31 July 2017 (19).

Main exclusion criteria in the cohorts were: (i) Inability to provide informed consent; (ii) Important lung disease potentially affecting LUS findings, such as pulmonary fibrosis, previous pneumectomy or lobectomy, pulmonary cancer or metastases; (iii) Other severe diseases hampering image acquisition.

Each cohort study was conducted in accordance with the Declaration of Helsinki and was approved by the respective Ethics Committees. Validation of the methodology has been performed and published previously (13).

Lung ultrasound and B-line count methods

LUS was performed by experienced cardiologists and resident doctors at each site using 28-zone scanning protocol in all cohorts except in the Siena and Barcelona cohort studies (15, 19), where the 8-zone method was used, as described below ***(Figure 1S***). Subsequently, we used the following method to calculate the sum of B-lines for 8-zones from 28-zone LUS results:

B-line count method (***Figure 1S***): we grouped the 28 zones of LUS (4) in eight zones (20) as follows:
I) parasternal and midclavicular zones in second and third intercostal space on right side (4 zones grouped in 1 zone).
II) parasternal and midclavicular zones in second and third intercostal space on left side (4 zones grouped in 1 zone).
III) anterior and mid-axillary zones in second and third intercostal space on right side (4 zones grouped in 1 zone).
IV) anterior and mid-axillary zones in second and third intercostal space on left side (4 zones grouped in 1 zone).

V) para-sternal and midclavicular zones in fourth and fifth intercostal space on right side (4 zones grouped in 1 zone).

VI) anterior and mid-axillary zones in fourth and fifth intercostal space on right side (4 zones grouped in 1 zone).

VII) para-sternal and midclavicular zones in fourth intercostal space on left side (2 zones grouped in 1 zone).

VIII) anterior and mid-axillary zones in fourth intercostal space on left side (2 zones grouped in 1 zone).

Thereafter, we summed the highest number of B-lines seen in one of the 4 or 2 zones of each group to obtain their total count.

Study outcome

The primary outcome of this study was a composite of re-hospitalization for HF and all-cause death and the secondary outcomes were components of the primary outcome. All patients were followed-up in each institution, and patients and their relatives were contacted regularly by email or phone to collect clinical events of interest.

Statistical analyses

We combined the patient-level data from above-mentioned cohorts to constitute admission cohort (AHF-core, Nancy, Siena and Pisa cohort), discharge cohort (AHF-core, Pisa, Siena and Perugia cohort) and out-patient cohort (Nis, Hull and Barcelona cohort). Categorical variables are presented as percentages and continuous variable are presented as mean ± standard deviation for normally distributed data and median, 25th and 75th percentiles for skewed data. For each cohort of admission, discharge and out-patients, patients were divided into 3 groups according to the tertiles of B-lines.

Time-to-event comparisons were analysed using log-rank test and Cox proportional hazards models. Survival probabilities were estimated using the Kaplan-Meier method and plotted as survival curves with cohort specific tertiles of B-line counts. Cox proportional-hazards models were then used to obtain crude and adjusted hazard ratios (HR). The Cox models were adjusted for age and sex in M1 model and for age, sex, eGFR, systolic blood pressure in M2 model and adjustment variables of M2 model and natriuretic peptides (Z score of BNP and NT-proBNP) in M3 model. Further, Cox models were adjusted for cohort as a factor because of differences between the cohorts. Further, we tested the association of outcome with B-lines while adjusting for E/e' on top of M3 model. To illustrate the association of tertiles of B-lines with outcome according to age, sex, LVEF and presence/absence of AF, forest plots were created for admission, discharge and out-patient cohorts. In addition, we assessed the association of tertiles of B-lines of upper and lower LUS zones with the primary outcome.

The incremental prognostic value of B-line counts on top of risk models (clinical/biological model, MAGGIC score and AHEAD score) was assessed by calculating continuous net reclassification improvement after adding total B-line counts to clinical/biological model, MAGGIC and AHEAD score. The clinical/biological model included age, sex, eGFR, systolic blood pressure, and natriuretic peptides (Z score of BNP and NT-pro-BNP). The MAGGIC score is based on 13 variables and predicts morbidity (21) and mortality in patients with HF (21, 22). The AHEAD score is a simple risk stratification tool for patients with HF based on 5 risk factors which are AF, haemoglobin <130 g/l for men and 120 g/l for women, elderly (age>70years), abnormal renal function (creatinine>130 µmol/l) and diabetes mellitus (23).

In addition, we conducted an intra-class correlation analysis to assess the inter-observer variability of the cardiologists involved in B-lines quantification in each centers (n=7) (24). The resulting intra-class correlation of the assessors of the B-lines in this study was 0.80 (95%CI 0.65 - 0.91) supporting a good reproducibility of the B-lines assessment across centers.

The two-tailed significance level was set at $p< 0.05$. All analyses were performed using SAS version 9.4.6 (SAS Institute Inc., Cary, NC) and R version 3.6.1 (2019-07-05).

Results

Baseline characteristics

In the admission (N= 578) and discharge cohort (N= 389), the median age was 78 years, 41% of the participants were women and approximately 45% participants had LVEF<40% (**Table 1)**. The number of B-lines (maximum number possible = 80) in admission cohort was ≤ 15 in tertile 1, 16-29 in tertile 2 and >29 in tertile 3; at discharge, B-lines were ≤ 11 in tertile 1, 12-22 in tertile 2 and >22 in tertile 3. In the out-patient cohort (N= 980), median age was 72 years, 31% of the participants were women and around 31% participants had LVEF< 40%. The number of B-lines was ≤ 1 in tertile 1, 2-6 in tertile 2 and >6 in tertile 3.

Overall, patients in tertile 2 and tertile 3 were older and more likely to have clinical signs of congestion and atrial fibrillation than those in tertile 1; they also had a higher median BNP/NT-pro-BNP, but LVEF was similar. Median AHEAD and MAGGIC score were both higher in patients with a higher number of B-lines in discharge and out-patient cohorts (all p-values <0.05), whereas, AHEAD score was also higher in tertile 2 and tertile 3 compared to tertile 1 in admission cohort.

Prognostic capacity of B-lines at admission, discharge and out-patient setting

Over a median follow-up of 180 (90-589), 126 (75 - 180) and 829 (527 - 968) days, the primary outcome occurred in 39% (n=226), 48% (n=188) and 30% (n=296) of patients in admission, discharge and out-patient cohorts, respectively. Higher B-line count was significantly associated with higher frequency of primary outcome in all the cohorts (All p-values < 0.001) (***Figure 1***).

In the discharge cohort, compared to tertile 1, tertile 2 and tertile 3 were significantly associated with increased risk in fully adjusted model (HR T2 vs T1: 1.80 (1.01 - 3.22), p=0.046 and T3 vs T1: 5.81 (3.30 - 10.25), p<0.0001)). When further adjusting on E/e', the association of higher

number of B-line counts with the primary outcome remained significant in the discharge cohort (tertile 1 vs tertile 2 and tertile 3 combined, HR: 3.26 (1.24 – 8.54), p= 0.016).

In the out-patient cohort, tertile 3 was significantly associated with increased risk in adjusted clinical model (HR = 3.42 (1.22 - 9.59)), however when the model was further adjusted with natriuretic peptides no association was observed between number of B-lines and outcome.

Further, higher B-line count was not associated with increased risk of hospitalization for HF or death at admission and in out-patient cohort in fully adjusted model whereas, higher B-line count at discharge (tertile 3 vs tertile 1) was associated with significantly increased risk of both, hospitalization for HF and death (***supplementary Table 2***).

In the admission and discharge cohorts, higher number of B-lines in upper zone (tertile 3 vs tertile 1) were significantly associated with increased risk of outcomes whereas higher number of B-lines in lower zone were less predictive. In contrast, a higher number of B-lines in upper zone and in lower zone were both significantly associated with increased risk in out-patient cohort with similar HR: (2.40 (1.12 - 5.13), p=0.024 and 2.55 (1.03 - 6.30), p= 0.043. respectively. (**Supplementary table 3**)

Association of B-lines with primary outcome according to clinical variables

In the admission cohort, the association of B-lines with primary outcome was affected by the underlying heart rhythm (p-interaction =0.0016) (***Figure 2***). In the discharge cohort, the association of B-lines with primary outcome was influenced by sex (p-interaction=0.007) whilst in the out-patient cohort, the prognostic capacity of B-lines was not influenced by any clinical variables (all p-interaction > 0.05).

Incremental prognostic value of risk scores by adding number of B-lines counts

In admission and discharge cohort, addition of B-lines to clinical, MAGGIC or AHEAD risk score significantly improved net reclassification for primary outcome (all p-values ≤ 0.01) ***(Figure 3).*** The net reclassification improvement was higher than 30% in the discharge cohort.

In out-patient cohort, addition of B-lines improved net reclassification of clinical model by 13.8% (p=0.03) and of AHEAD score by 18.9 % (p=0.03) however, the net reclassification improvement was non-significant over MAGIC score.

Discussion

In this pooled cohort study, we found that higher B-line counts were independently associated with the risk of hospitalization for HF and death in both in-patient and in an out-patient setting. Further, LUS predicted outcome irrespective of the clinical characteristics. Moreover, addition of B-lines to clinical, MAGGIC and AHEAD scores improved risk stratification. AF influenced the prognostic capacity of B-lines in the admission cohort but not in discharge and out-patient cohorts.

Prognostic capacity of LUS in hospitalised patients and in out-patients

In a very heterogeneous group of patients with HF, higher count of B-lines predicts mid-term risk of hospitalisation or death in different settings. The association was, however, not significant when adjusting on NP in outpatients, possibly because of limited statistical power in this relatively low risk subgroup. Similar results were reported by Platz et al., where pre-discharge higher tertiles of B-lines were associated with higher risk at short- and mid-term outcomes in patients with HF (24). Moreover, post-hoc analysis of ESCAPE trial demonstrated that persistent pulmonary congestion was associated worse prognostic outcome within 6 months of hospitalization for HF and management of congestion may be more prognostically important than improving cardiac index in these patients (25). However, no significant association between the number of B-lines at admission and outcome was identified despite a sizeable sample size (N=578) and high risk of events in this subgroup. Our results are in line with other previous reports that assessed pulmonary congestion using chest radiography on admission and found only weak and inconsistent associations with future risk (26, 27). In contrast, discharge congestion, referred to as “residual congestion” (16), is probably the key prognostic variable, capturing the ability to decongest patients during the hospitalization which probably parallels the ability to maintain decongestion thereafter. Moreover, a recent study shows that poor in-

hospital improvement in congestion is associated with poor prognosis (28). These findings indicate that a patient may still have good prognosis despite higher level of congestion if the patients responds well to the given treatment. In addition, previous studies have reported conflicting results on the prediction capacity of B-lines measured at admission (3, 29). In a study by Gargani et al., more than 50 B-lines at admission were not associated with increased risk in patients with acute HF (aHR: 4.87 (0.88 – 27.06), p= 0.07) (3) whereas, in another study higher number of B-lines (>30) was associated in adverse outcome in overall population. (29) Taken together, this suggests that residual pulmonary congestion at the time of discharge, rather than congestion at admission, is one of the major factors associated with a poor prognosis in HF. Our findings highlight the limited prognostic value of admission congestion rather than the limited value of LUS itself.

Taking advantage of the individual patient data, we found that the prognostic capacity of LUS to identify patients at a higher risk remains mostly unchanged in the sub-group analysis according to age, sex, LVEF, natriuretic peptide levels and presence/absence of AF. These results suggest that the prognostic value of B-lines is unaffected by clinical characteristics, something that is not necessarily true for other echocardiographic indices of congestion (5, 15, 30). For instance, in patients with AF, E/A ratio and E/e' cannot be reliably measured or used to predict future risk (15).

B-lines on top of clinical risk scores for predicting risk of outcome

Another important finding of this study is that adding B-line count to clinical risk scores, like MAGGIC and AHEAD, improved the net reclassification of patients at high risk regardless of the clinical setting. Our findings concur with the previously published study, which assessed incremental capacity of B-lines on top of 4 different HF risk scores in a cohort of 123 AHF patients at discharge. The study reported that addition of B-line count significantly improved the predictive value of HF risk scores (31). This improvement is likely related to the detection of subclinical congestion not identified on physical examination. In stable HF patients, up to 50% of patients have sub-clinical congestion that can go undetected on physical examination at the time of discharge or in an out-

patient setting, unless ultrasound is used (18, 32), and that worsening subclinical congestion by ultrasound is associated with higher natriuretic peptides and an increased risk of cardiovascular events. Hence, LUS can improve the ability of AHEAD or MAGGIC score to identify the patients at higher risk. In addition, LUS significantly improved net reclassification in a clinical/biological model that included powerful prognosticators such as natriuretic peptides. This may be related to LUS specifically evaluating pulmonary congestion, which is intrinsically related to dyspnoea, whereas natriuretic peptides are possibly related to less specific congestion patterns and influenced by renal function and the presence of AF (13).

Clinical implications

Congestion assessment using LUS can provide important prognostic information in patients with HF irrespective of the phenotype and clinical setting (33). LUS can be implemented systematically along with clinical examination at the time of discharge and during follow-up visits to identify the patients at higher risk, especially those who do not have clinical signs of congestion (18). Nowadays, it is becoming easy and practical to conduct LUS in any setting with the availability of hand-held devices and transducers that can be easily connected to mobile devices, including smartphones. Moreover, natriuretic peptides assessment is not always available across all hospitals or to many primary care providers; and when it is, it will take time to obtain results (9). Findings from LUS can be interpreted in real time and serial examinations can quantify and monitor response to treatment, particularly diuretics (34). Although results were disappointing for LUS-guided management in hospitalised patients (35), LUS-guided management after discharge was associated with clinical benefits in several clinical trials in patients with HF (36, 37). Recent studies demonstrate that novice residents with limited or no previous experience in LUS can acquire proficiency in reliably quantifying B-lines with minimal training (38, 39). Moreover, LUS can be easily performed by nurses and other healthcare providers, which reinforces the incentive to use LUS for assisting with management in this context (40).

Limitations

Our study has some limitations. First, although this is a patient-level pooled analysis of eight cohorts, the admission, discharge and out-patient cohorts were moderate in size limiting the generalisability of the results. Second, individual cohorts had some differences in available baseline data, method of variable measurements and patient selection. Third, experienced practitioners in each institution assessed LUS; however, there could have been inter-observer variability. Further, B-line counts could be different depending on cohort studies, possibly because of the different LUS protocol (patient position, transducer settings) of the cohort studies. Despite these differences, we found strong association of B-lines with outcomes in discharge and out-patient cohorts, adding to generalisability of our results. LUS findings are pragmatically relevant in a broad array of clinical settings and centres. Fourth, in the Nancy cohort and AHF-core, patients underwent LUS assessment within 3 days from their admission, whereas in Siena and Pisa patient underwent LUS within 12 hours and 24 hours from admission, respectively: it is possible that prior administration of diuretic therapy may have influenced B-line counts in these patients.

Conclusion

In this study, a higher number of B-lines in patients with HF was associated with increased risk of morbidity and mortality and improved risk stratification, regardless of the clinical setting or patients' profile.

Table 1: Baseline characteristics according to the tertiles of B-lines in highest number of B-lines per zone method in each clinical setting

	In patients - Admission							In patients - Discharge							Out-patients						
Max counts = 80	**Tertile 1 (Counts <=15) (n=204)**		**Tertile 2 (Counts 16-29) (n=190)**		**Tertile 3 (Counts >29) (n=184)**		**P-value**	**Tertile 1 (Counts <=11) (n=140)**		**Tertile 2 (Counts 12-22) (n=123)**		**Tertile 3 (Counts >22) (n=126)**		**P-value**	**Tertile 1 (Counts <=1) (n=319)**		**Tertile 2 (Counts 2-6) (n=341)**		**Tertile 3 (Counts >6) (n=320)**		**P-value**
	n	**median (IQR)/n (%)**	**n**	**median (IQR)/n (%)**	**n**	**median (IQR)/n (%)**		**n**	**median (IQR)/n (%)**	**n**	**median (IQR)/n (%)**	**n**	**median (IQR)/n (%)**		**n**	**median (IQR)/n (%)**	**n**	**median (IQR)/n (%)**	**n**	**median (IQR)/n (%)**	
Age, yrs	204	74.0 (66.5 - 80.0)	190	77.5 (70.0 - 82.0)	184	79.0 (75.0 - 85.0)	< 0.0001	140	75.0 (67.0 - 80.0)	123	80.0 (75.0 - 85.0)	126	80.5 (76.0 - 86.0)	< 0.0001	319	67.5 (58.2 - 75.0)	341	71.0 (62.1 - 78.9)	320	76.4 (69.0 - 82.3)	< 0.0001
Women, N (%)	204	77 (37.7 %)	190	80 (42.1 %)	184	82 (44.6 %)	0.38	140	44 (31.4 %)	123	65 (52.8 %)	126	53 (42.1 %)	0.002	319	99 (31.0 %)	341	114 (33.4 %)	320	96 (30.0 %)	0.62
Body mass index, kg/m²	183	27.3 (24.1 - 30.5)	162	29.0 (26.0 - 30.9)	180	26.0 (24.0 - 28.7)	< 0.0001	98	29.0 (26.0 - 31.8)	110	28.3 (25.0 - 30.5)	119	26.0 (24.4 - 28.4)	< 0.0001	318	28.4 (25.2 - 32.5)	340	28.4 (25.3 - 31.2)	315	27.1 (24.1 - 30.2)	0.0006
Medical history, N (%)																					
Ischemic heart disease	204	83 (40.7 %)	190	88 (46.3 %)	183	95 (51.9 %)	0.088	140	59 (42.1 %)	122	54 (44.3 %)	126	61 (48.4 %)	0.59	319	128 (40.1 %)	340	153 45.0 %)	320	154 48.1 %)	0.12
Atrial fibrillation	188	63 (33.5 %)	181	64 (35.4 %)	174	44 (25.3 %)	0.093	127	43 (33.9 %)	118	24 (20.3 %)	118	40 (33.9 %)	0.029	314	58 (18.5 %)	339	90 (26.5 %)	316	140 44.3 %)	< 0.0001
Prior HF	198	172 (86.9 %)	99	87 (87.9 %)	59	54 (91.5 %)	0.68	108	88 (81.5 %)	34	27 (79.4 %)	31	27 (87.1 %)	0.75	24	20 (83.3 %)	37	30 (81.1 %)	0	0 (0 %)	1
COPD	201	28 (13.9 %)	98	14 (14.3 %)	57	6 (10.5 %)	0.83	107	34 (31.8 %)	34	8 (23.5 %)	30	5 (16.7 %)	0.23	319	33 (10.3 %)	341	43 (12.6 %)	320	57 (17.8 %)	0.02
CRT	190	48 (25.3 %)	90	21 (23.3 %)	49	19 (38.8 %)	0.12	95	20 (21.1 %)	29	2 (6.9 %)	23	11 (47.8 %)	0.003	295	52 (17.6 %)	304	28 (9.2 %)	320	45 (14.1 %)	0.009
Clinical examination																					
NYHA III/IV, N (%)	195	95 (48.7 %)	93	61 (65.6 %)	51	37 (72.5 %)	0.001	54	3 (5.6 %)	13	6 (46.2 %)	7	3 (42.9 %)	0.0003	319	34 (10.7 %)	341	46 (13.5 %)	320	100 31.3 %)	< 0.0001
Systolic BP, mmHg	193	130 (115 - 143)	177	125 (110 - 140)	182	130 (110 - 140)	0.19	55	115 (105 - 127)	17	105 (103 - 111)	14	115 (95- 120)	0.23	101	131 (121 - 147)	147	130 (119 - 146)	155	136 (122 - 159)	0.011
Heart rate, bpm	192	72 (64 - 87)	176	87 (78 - 95)	182	89 (85 - 96)	< 0.0001	55	74 (65 - 80)	17	80 (70 - 90)	14	75 (70 - 82)	0.26	101	69 (60- 80)	147	70 (64 - 80)	155	73 (65 - 81)	0.14
Leg edema, N (%)	200	61 (30.5 %)	190	109 (57.4 %)	184	114 (62.0 %)	< 0.0001	87	11 (12.6 %)	106	22 (20.8 %)	109	49 (45.0 %)	< 0.0001	101	20 (19.8 %)	147	39 (26.5 %)	155	79 (51.0 %)	< 0.0001
Rales, N (%)	201	72 (35.8 %)	190	146 (76.8 %)	184	155 (84.2 %)	< 0.0001	87	15 (17.2 %)	106	22 (20.8 %)	109	70 (64.2 %)	< 0.0001	101	0 (0.0 %)	147	7 (4.8 %)	155	35 (22.6 %)	< 0.0001
Medications, N(%)																					
ACEi or ARB	182	127 (69.8 %)	178	132 (74.2 %)	175	149 (85.1%)	0.002	110	79 (71.8 %)	117	92 (78.6 %)	122	97 (79.5 %)	0.34	319	281 (88.1 %)	341	290 (85.0%)	320	245 (76.6%)	0.0004
Beta-blocker	182	60 (33.0 %)	178	80 (44.9 %)	175	100 (57.1%)	< 0.0001	110	57 (51.8 %)	117	66 (56.4 %)	122	71 (58.2 %)	0.6	319	296 (92.8 %)	341	304 (89.1%)	320	275 (85.9%)	0.018
MRA	181	81 (44.8 %)	88	39 (44.3 %)	50	27 (54.0 %)	0.49	78	43 (55.1 %)	28	13 (46.4 %)	27	15 (55.6 %)	0.74	319	175 (54.9 %)	341	171 (50.1%)	320	153 (47.8%)	0.19
Loop diuretics	182	147 (80.8 %)	178	164 (92.1 %)	175	158 (90.3%)	0.003	110	104 (94.5)	117	105 (89.7%)	122	115 (94.3 %)	0.32	319	215 (67.4 %)	341	231 (67.7%)	320	255 (79.7%)	0.0003
Laboratory findings																					
Hemoglobin, g/dl	131	12.8 (11.6 - 14.0)	159	12.2 (10.8 - 13.6)	168	12.0 (10.9 - 13.3)	0.001	99	12.6 (11.1 - 13.9)	114	11.8 (10.5 - 13.6)	114	11.5 (10.2 - 12.7)	0.002	318	13.8 (12.6 - 14.9)	341	13.5 (12.3 - 14.5)	318	13.1 (12.0 - 14.1)	< 0.0001
eGFR, ml/min/1.73m²	197	60.9 (47.0 - 75.9)	189	50.8 (35.4 - 72.0)	181	48.6 (37.2 - 63.1)	< 0.0001	111	53.0 (38.8 - 66.5)	116	41.4 (30.1 - 59.1)	120	45.6 (32.9 - 57.8)	0.005	319	69.7 (50.2 - 87.7)	339	64.4 (48.2 - 81.1)	319	57.7 (41.2 - 77.0)	< 0.0001
BNP, pg/ml	105	498 (252 - 795)	153	822 (576 - 1196)	154	1230 (790 - 1870)	< 0.0001	90	342 (177 - 616)	111	471 (312 - 746)	111	988 (714 - 1780)	< 0.0001	24	41 (32 - 55)	37	71 (45 - 127)	0	-	0.0003
NT pro-BNP, pg/ml	147	1664 (907 - 2981)	57	3441 (1740 - 5588)	47	4632 (2851 - 9134)	< 0.0001	41	1655 (572 - 3019)	15	7474 (1934 - 10475)	20	2344 (1820 - 7440)	0.0002	288	460 (187 - 1251)	295	783 (293 - 1740)	316	1599 (735 - 3419)	< 0.0001
Echocardiography																					
LVEF, %	204	40.0 (30.0 - 53.5)	189	40.0 (30.0 - 50.0)	183	38.0 (25.0 - 50.0)	0.38	140	40.0 (30.0 - 50.0)	123	40.0 (32.0 - 52.0)	125	40.0 (25.0 - 50.0)	0.077	319	47.0 (36.0 - 55.0)	341	47.0 (36.0 - 57.0)	320	45.0 (36.0 - 55.0)	0.56
LVEF<40%	204	97 (47.5 %)	189	84 (44.4 %)	183	93 (50.8 %)	0.48	140	64 (45.7 %)	123	52 (42.3 %)	125	60 (48.0 %)	0.67	319	101 (31.7 %)	341	108 (31.7%)	320	102 (31.9%)	1.00
Blines	204	6.0 (0.0 - 11.0)	190	24.5 (20.0 - 27.0)	184	36.0 (32.0 - 42.0)	< 0.0001	140	6.0 (3.0 - 9.0)	123	16.0 (14.0 - 20.0)	126	29.0 (26.0 - 34.0)	< 0.0001	319	0.0 (0.0 - 1.0)	341	3.0 (2.0 - 5.0)	320	12.0 (8.0 - 18.0)	<0.0001
Scores																					
AHEAD score	204	1.5 (1.0 - 2.0)	190	2.0 (1.0 - 3.0)	184	2.0 (2.0 - 3.0)	< 0.0001	140	2.0 (1.0 - 3.0)	123	2.0 (2.0 - 3.0)	126	3.0 (2.0 - 3.0)	< 0.0001	319	1.0 (0.0 - 2.0)	341	2.0 (1.0 - 2.0)	320	2.0 (1.0 - 3.0)	< 0.0001

MAGGIC score	204	22.0 (18.0 - 27.0)	190	22.0 (19.0 - 25.0)	184	22.0 (20.0 - 27.0)	0.26	140	18.0 (14.0 - 22.0)	123	21.0 (18.0 - 24.0)	126	22.0 (19.0 - 24.0)	< 0.0001	319	14.0 (9.0 - 20.0)	341	17.0 (12.0 - 22.0)	320	21.0 (16.0 - 26.0)	< 0.0001

ACEi: ACE inhibitor; ARB: angiotensin receptor blocker; COPD: chronic obstructive pulmonary disease; CRT: cardiac resynchronization therapy; HF: heart failure; MRA: mineralocorticoid receptor antagonist; LVEF: left ventricular ejection fraction

Figure 1: Crude and adjusted hazard ratios for association between B-lines and primary outcome for B-line count

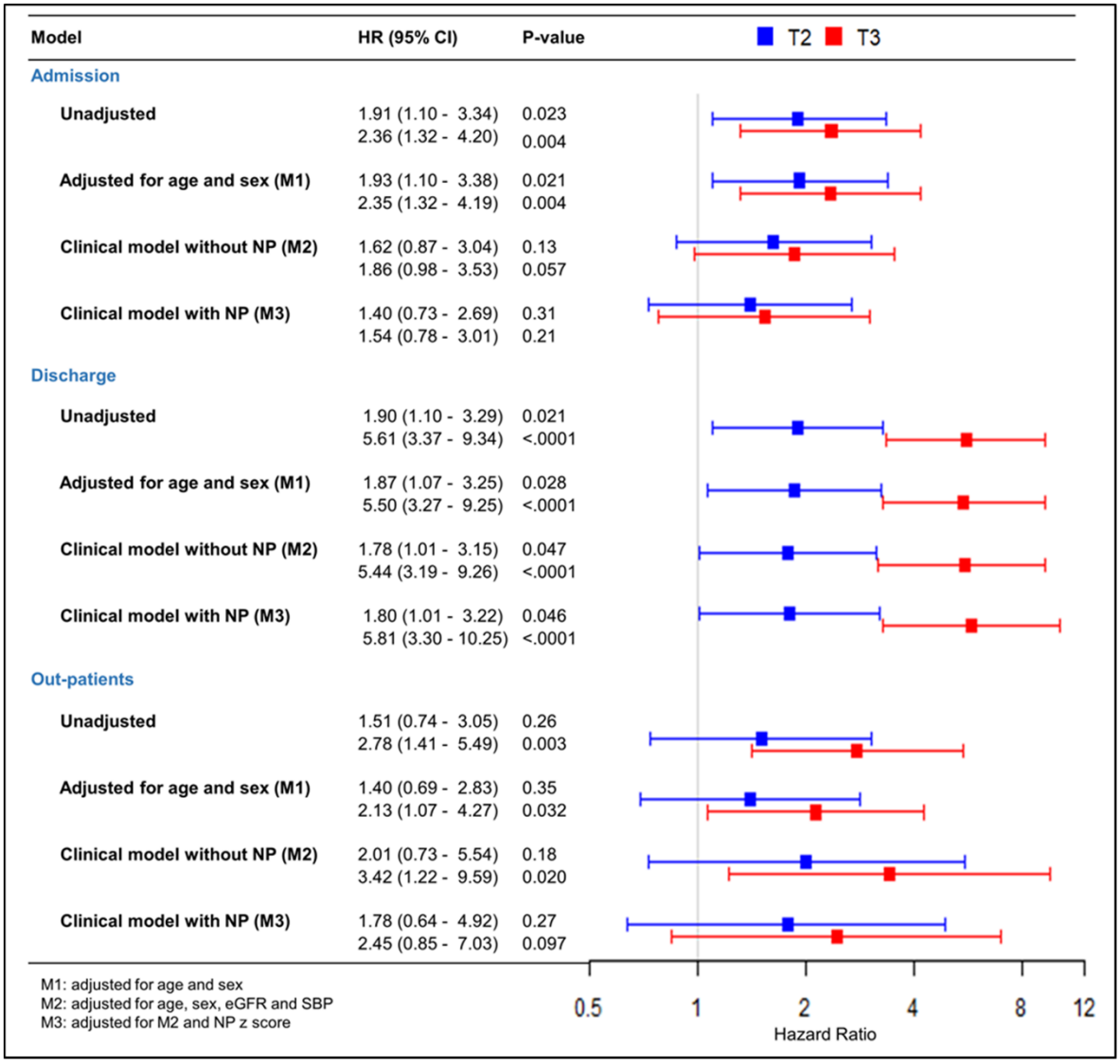
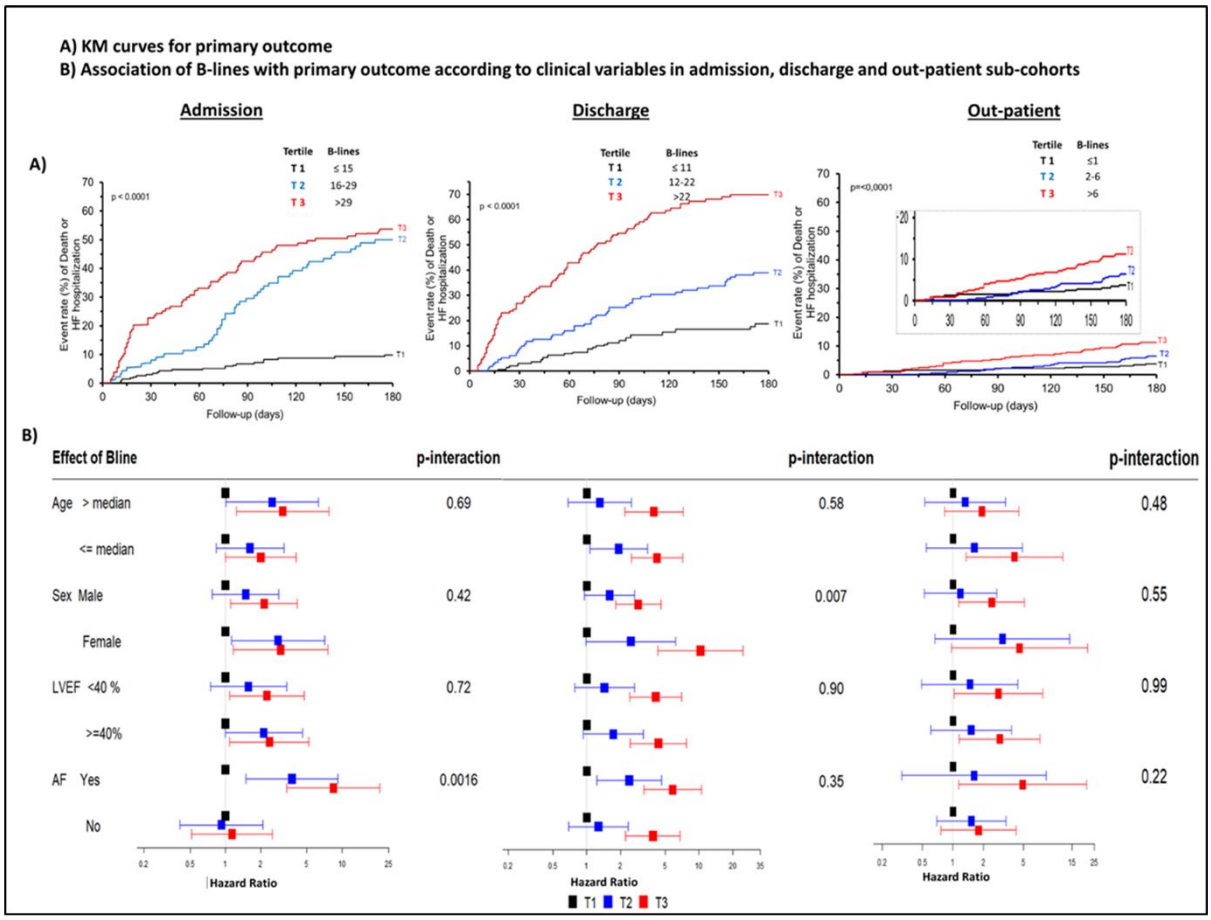
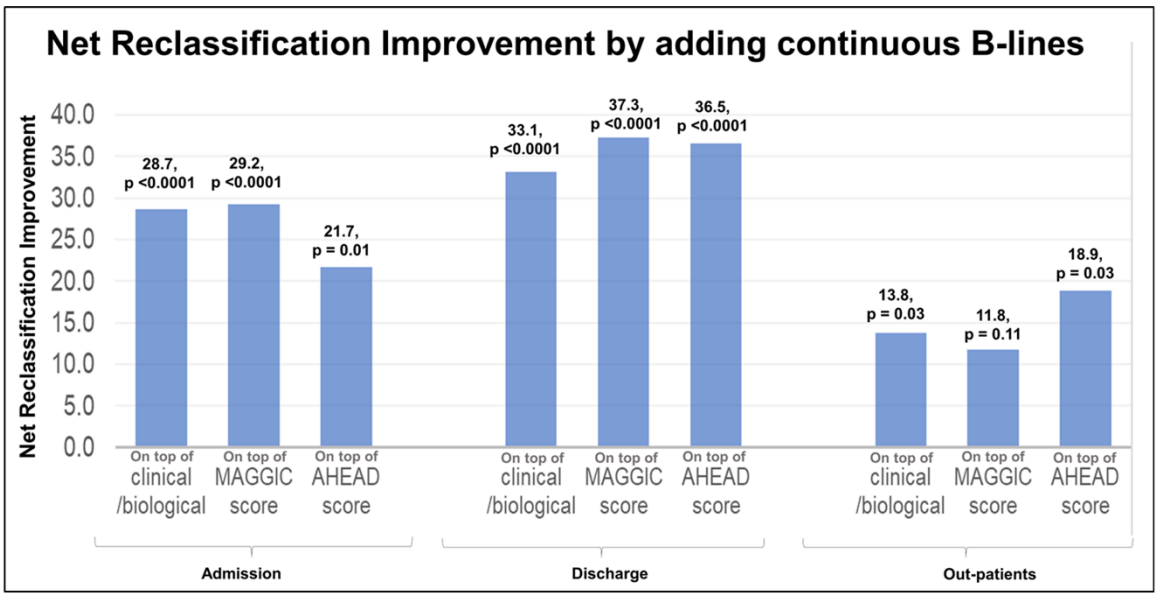


Figure 2: (a) KM curves for primary outcome and (b) association of B-lines with primary outcome according to clinical variables in admission, discharge and out-patient cohorts



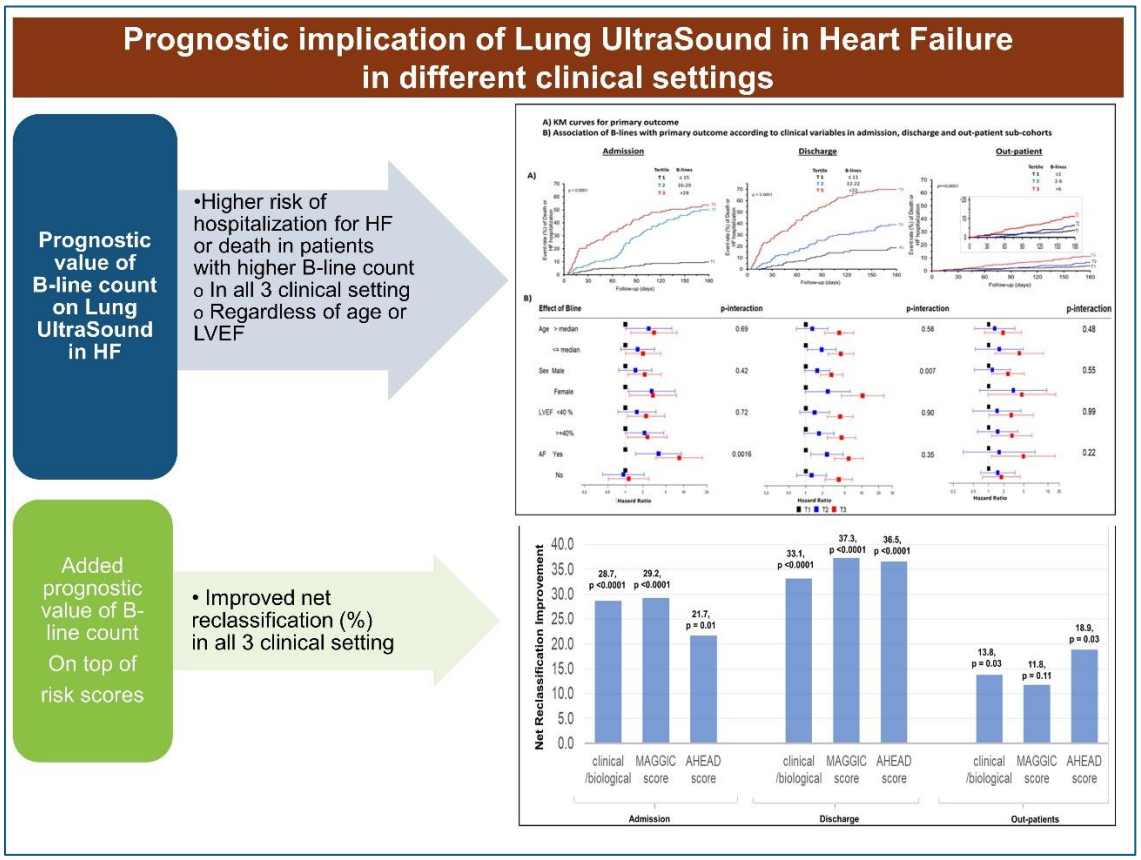
Inset: magnified view of KM curves in the plot for out-patients.

Figure 3: Net Reclassification Improvement for risk of primary outcome in clinical model, MAGGIC score and AHEAD score by adding continuous B-lines



Legends on the bar present the net reclassification improvement and the p-value.

Figure: Graphical abstract



Data availability statement:

The data that supports the findings of this study are available on reasonable request to corresponding authors.

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Supplementary data

Supplementary Table 1: Patients Characteristics in each cohort*

	AHF-Core	AHF-Core	Nancy	Pisa	Pisa	Siena	Siena	Perugia	Nis	Hull	Barcelona
N	**30**	**30**	**50**	**307**	**307**	**216**	**216**	**60**	**61**	**342**	**577**
AHF or CHF	**AHF**	**AHF**	**AHF**	**AHF**	**AHF**	**AHF**	**AHF**	**AHF**	**CHF**	**CHF**	**CHF**
timing of AHF	**Admission**	**Discharge**	**Admission**	**Admission**	**Discharge**	**Admission**	**Discharge**	**Discharge**			
Age, yrs	79.6 ± 7.3	—	73.4 ± 10.5	70.8 ± 11.0	—	80.5 ± 7.7	—	72.2 ± 10.3	64.7 ± 8.9	73.4 ± 11.5	68.8 ± 12.3
Women, N (%)	6 (20.0 %)	—	31 (62.0 %)	94 (30.6 %)	—	114 (52.8 %)	—	19 (31.7 %)	34 (55.7 %)	113 (33.0 %)	162 (28.1 %)
Body mass index, kg/m²	28.8 ± 4.8	—	—	27.2 ± 4.8	—	27.7 ± 3.3	—	—	29.5 ± 3.7	29.7 ± 6.2	27.9 ± 5.0
Medical history, N (%)											
Hypertension	26 (86.7 %)	—	—	—	—	135 (62.5 %)	—	—	—	188 (55.0 %)	334 (57.9 %)
Diabetes	12 (40.0 %)	—	—	—	—	93 (43.1 %)	—	—	—	99 (28.9 %)	219 (38.0 %)
Ischemic heart disease	13 (44.8 %)	—	31 (62.0 %)	139 (45.3 %)	—	94 (43.5 %)	—	19 (31.7 %)	18 (29.5 %)	167 (48.8 %)	250 (43.4 %)
Atrial fibrillation	—	—	34 (75.6 %)	91 (29.6 %)	—	54 (25.0 %)	—	28 (46.7 %)	10 (16.4 %)	164 (48.0 %)	114 (20.1 %)
Prior HF	16 (53.3 %)	—	34 (75.6 %)	286 (93.8 %)	—	—	—	45 (75.0 %)	50 (82.0 %)	0 (0.0 %)	—
COPD	5 (18.5 %)	—	8 (16.7 %)	39 (12.8 %)	—	—	—	22 (36.7 %)	3 (4.9 %)	66 (19.3 %)	64 (11.1 %)
CRT	0 (0.0 %)	—	0 (0.0 %)	93 (30.5 %)	—	—	—	8 (13.3 %)	0 (0.0 %)	28 (8.2 %)	97 (16.8 %)
Clinical examination											
NYHA III/IV, N (%)	6 (85.7 %)	—	42 (84.0 %)	160 (52.1 %)	—	—	—	9 (15.0 %)	0 (0.0 %)	85 (24.9 %)	95 (16.5 %)
Systolic BP, mmHg	117.8 ± 17.5	118.4 ± 16.5	119.6 ± 22.4	128.7 ± 24.5	—	130.1 ± 17.8	—	111.9 ± 16.0	123.9 ± 8.4	139.1 ± 24.6	—
Heart rate, bpm	74.0 ± 13.6	72.4 ± 13.3	95.3 ± 16.5	79.4 ± 20.6	—	91.2 ± 6.6	—	77.0 ± 13.9	74.8 ± 7.2	72.0 ± 14.0	—
Leg edema, N (%)	22 (75.9 %)	13 (50.0 %)	24 (48.0 %)	91 (30.1 %)	—	156 (72.2 %)	58 (26.9 %)	11 (18.3 %)	21 (34.4 %)	117 (34.2 %)	—
Rales, N (%)	24 (82.8 %)	14 (53.8 %)	41 (82.0 %)	125 (41.3 %)	—	198 (91.7 %)	75 (34.7 %)	18 (30.0 %)	2 (3.3 %)	40 (11.7 %)	—
Medication, N (%)											
ACEi or ARB	20 (66.7 %)	19 (65.5 %)	42 (84.0 %)	188 (71.5 %)	—	177 (81.9 %)	—	38 (63.3 %)	58 (95.1 %)	290 (84.8 %)	468 (81.1 %)
Beta-blocker	21 (70.0 %)	21 (72.4 %)	23 (46.0 %)	81 (30.8 %)	—	126 (58.3 %)	—	36 (60.0 %)	61 (100 %)	287 (83.9 %)	527 (91.3 %)
MRA	7 (23.3 %)	8 (27.6 %)	2 (4.0 %)	155 (58.9 %)	—	—	—	36 (60.0 %)	6 (9.8 %)	166 (48.5 %)	327 (56.7 %)
Loop diuretics	24 (80.0 %)	—	50 (100 %)	220 (83.7 %)	—	198 (91.7 %)	—	60 (100 %)	51 (83.6 %)	258 (75.4 %)	392 (67.9 %)
Laboratory findings											
Hemoglobin, g/dl	12.0 ± 2.1	12.1 ± 2.5	12.5 ± 2.0	12.6 ± 1.9	13.0 ± 4.6	12.2 ± 2.0	12.0 ± 2.0	12.1 ± 2.4	13.6 ± 1.3	13.1 ± 1.7	13.5 ± 1.6
Sodium, mmol/l	141.5 ± 3.5	140.8 ± 4.8	138.1 ± 3.7	138.8 ± 22.2	132.4 ± 14.6	137.7 ± 4.3	137.5 ± 4.1	140.8 ± 3.7	143.8 ± 3.1	—	—
Creatinine, mg/dl	1.6 ± 0.7	1.6 ± 0.6	1.5 ± 1.0	1.4 ± 0.7	1.7 ± 0.7	1.4 ± 0.5	1.6 ± 0.6	1.3 ± 0.5	1.0 ± 0.3	1.3 ± 0.5	1.3 ± 0.8
BNP, pg/ml	1489 (717 - 2106)	1568 (524 - 2015)	765 (545 - 1769)	500 (266 - 898)	328 (183 - 844)	991 (727 - 1598)	586 (339 - 981)	576 (234 - 1142)	57 (39 - 94)	—	—
NT pro-BNP, pg/ml	4753 (3458 - 26225)	4661 (2228 - 11393)	—	2287 (1209 - 4938)	1841 (869 - 4447)	—	—	—	—	1276 (461 - 2649)	722 (262 - 1760)
Echocardiography											
LVEF, %	42.8 ± 14.5	40.9 ± 12.8	36.1 ± 13.8	38.6 ± 14.3	—	41.5 ± 12.2	41.5 ± 12.2	40.7 ± 16.7	57.8 ± 6.5	45.1 ± 13.6	45.5 ± 12.6

LVEF<40%	12 (42.9 %)	11 (44.0 %)	26 (52.0 %)	169 (55.0 %)	—	83 (38.4 %)	—	33 (55.0 %)	0 (0.0 %)	124 (36.3 %)	187 (32.4 %)
Lung echo											
B-lines (8 sites) supero medial counts	9.0 (4.0 - 15.0)	5.5 (1.0 - 10.0)	11.0 (8.0 - 18.0)	5.0 (0.0 - 12.0)	3.0 (0.0 - 7.0)	—	—	3.5 (2.0 - 10.0)	0.0 (0.0 - 1.0)	2.0 (0.0 - 5.0)	—
B-lines (8 sites) Highest count within a zone	22.5 (8.0 - 34.0)	13.0 (5.0 - 27.0)	17.0 (14.0 - 25.0)	12.0 (3.0 - 23.0)	8.0 (4.0 - 16.0)	31.0 (26.0 - 37.5)	22.0 (14.5 - 28.0)	5.0 (4.0 - 14.0)	2.0 (1.0 - 2.0)	6.0 (2.0 - 13.0)	3.0 (1.0 - 7.0)
Scores											
AHEAD score	2.3 ± 0.9	2.0 ± 0.9	2.1 ± 1.2	1.9 ± 1.0	2.0 ± 0.9	2.5 ± 1.0	2.7 ± 1.0	1.9 ± 1.1	0.6 ± 0.7	2.0 ± 1.2	1.6 ± 1.3
MAGGIC score	23.2 ± 6.2	19.1 ± 8.4	22.8 ± 6.2	22.7 ± 7.2	16.3 ± 5.5	21.6 ± 4.5	20.9 ± 4.1	19.8 ± 7.0	10.0 ± 4.1	19.8 ± 6.7	16.9 ± 7.3

ACEi: ACE inhibitor; ARB: angiotensin receptor blocker; COPD: chronic obstructive pulmonary disease; CRT: cardiac resynchronization therapy; HF: heart failure; MRA: mineralocorticoid receptor antagonist; LVEF: left ventricular ejection fraction

*For categorical variables, prevalence (number and %) is reported based on the frequency of measurements/observations available, and not on the number of patients enrolled in each cohort

Supplementary Table 2: Crude and adjusted hazard ratios for association between B-lines and secondary outcomes

	Death		HF hospitalisation	
Model	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**
Admission				
Unadjusted	2.32 (0.97 - 5.53)	0.058	1.86 (0.97 - 3.56)	0.060
	3.52 (1.45 - 8.50)	0.005	2.08 (1.05 - 4.12)	0.035
Adjusted for age and sex (M1)	2.36 (0.99 - 5.63)	0.053	1.85 (0.96 - 3.55)	0.064
	3.47 (1.44 - 8.41)	0.006	2.08 (1.05 - 4.12)	0.036
Clinical model without NP (M2)	2.03 (0.73 - 5.66)	0.18	1.52 (0.75 - 3.08)	0.24
	2.87 (1.02 - 8.05)	0.045	1.50 (0.72 - 3.11)	0.28
Clinical model with NP (M3)	1.95 (0.65 - 5.87)	0.23	1.31 (0.64 - 2.68)	0.47
	2.59 (0.85 - 7.93)	0.096	1.24 (0.58 - 2.64)	0.58
Discharge				
Unadjusted	2.46 (1.09 - 5.56)	0.031	1.93 (1.00 - 3.74)	0.051
	7.77 (3.65 - 16.55)	<0.0001	4.56 (2.45 - 8.47)	<0.0001
Adjusted for age and sex (M1)	2.30 (1.01 - 5.21)	0.047	2.01 (1.02 - 3.96)	0.043
	7.14 (3.33 - 15.35)	<0.0001	4.73 (2.49 - 8.97)	<0.0001
Clinical model without NP (M2)	1.86 (0.80 - 4.30)	0.15	1.98 (0.99 -3.98)	0.055
	6.26 (2.90 -13.54)	<.0001	4.84 (2.50 -9.37)	<.0001
Clinical model with NP (M3)	1.84 (0.80 - 4.26)	0.15	1.97 (0.98 -3.96)	0.057
	6.85 (3.11 - 15.11)	<0.0001	5.11 (2.58 -10.11)	<.0001
Out-patients				
Unadjusted	3.05 (0.63 - 14.73)	0.16	1.31 (0.59 - 2.88)	0.51
	7.06 (1.63 - 30.65)	0.009	2.23 (1.02 - 4.91)	0.046
Adjusted for age and sex (M1)	2.80 (0.58 - 13.51)	0.20	1.23 (0.55 - 2.72)	0.61
	5.25 (1.19 - 23.16)	0.028	1.81 (0.81 - 4.06)	0.15
Clinical model without NP (M2)	1.98 (0.99 - 3.98)	0.055	1.50 (0.52 - 4.34)	0.45
	4.84 (2.50 - 9.37)	<.0001	2.55 (0.83 - 7.85)	0.10
Clinical model with NP (M3)	No event in T1		1.24 (0.43 - 3.61)	0.69
			1.62 (0.50 - 5.22)	0.42

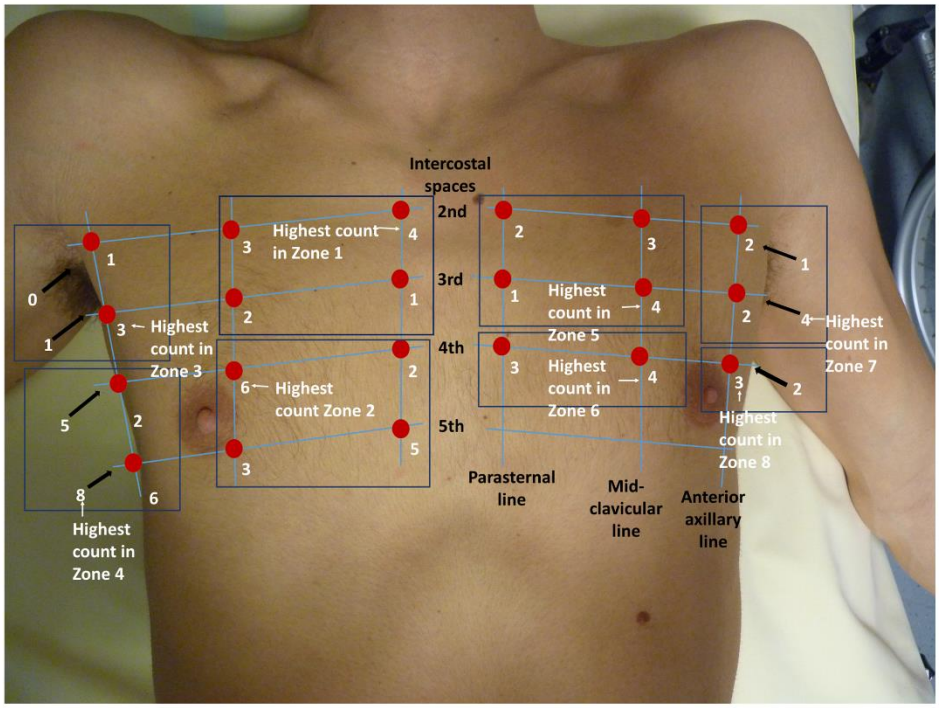
Supplementary table 3: Crude and Adjusted Hazard ratios for associations between B-lines upper and lower zone and primary outcomes

		Lower zone		Upper zone	
model	**B-Lines tertiles**	**HR (95 %CI)**	**P-value**	**HR (95 %CI)**	**P-value**
Admission					
Unadjusted	T2	0.91 (0.44 - 1.92)	0.81	1.58 (0.73 - 3.42)	0.24
	T3	1.80 (0.92 - 3.52)	0.084	**2.91 (1.47 - 5.75)**	**0.002**
Adjusted for sex and age (M1)	T2	0.93 (0.44 - 1.97)	0.86	1.61 (0.75 - 3.48)	0.23
	T3	1.81 (0.92 - 3.54)	0.086	**2.93 (1.47 - 5.83)**	**0.002**
Clinical model without NP (M2)	T2	0.99 (0.44 - 2.19)	0.97	1.42 (0.61 - 3.27)	0.41
	T3	1.17 (0.53 - 2.61)	0.7	2.14 (0.98 - 4.67)	0.056
Clinical model with NP (M3)	T2	0.85 (0.37 - 1.99)	0.71	1.38 (0.57 - 3.33)	0.47
	T3	1.02 (0.44 - 2.34)	0.97	1.91 (0.84 - 4.34)	0.12
Discharge					
Unadjusted	T2	1.85 (0.78 - 4.38)	0.16	1.63 (0.64 - 4.18)	0.31
	T3	**2.68 (1.20 - 6.00)**	**0.016**	**4.52 (2.07 - 9.90)**	**0.0002**
Adjusted for sex and age (M1)	T2	1.83 (0.77 - 4.39)	0.17	1.64 (0.64 - 4.24)	0.3
	T3	**2.60 (1.15 - 5.90)**	**0.022**	**4.63 (2.04 - 10.49)**	**0.0002**
Clinical model without NP (M2)	T2	1.64 (0.65 - 4.16)	0.30	1.97 (0.73 - 5.34)	0.18
	T3	2.40 (0.99 - 5.81)	0.053	**4.46 (1.79 - 11.10)**	**0.001**
Clinical model with NP (M3)	T2	1.73 (0.67 - 4.44)	0.25	2.00 (0.74 - 5.41)	0.17
	T3	2.43 (1.00 - 5.89)	0.0502	**4.39 (1.76 - 10.93)**	**0.002**
Out-patients					
Unadjusted	T2	1.52 (0.78 - 2.94)	0.22	1.55 (0.79 - 3.03)	0.2
	T3	**2.54 (1.43 - 4.51)**	**0.001**	**2.66 (1.42 - 4.98)**	**0.002**
Adjusted for sex and age (M1)	T2	1.43 (0.74 - 2.78)	0.29	1.54 (0.78 - 3.02)	0.21
	T3	**2.00 (1.11 - 3.61)**	**0.021**	**2.05 (1.08 - 3.88)**	**0.028**
Clinical model without NP (M2)	T2	1.68 (0.73 - 3.87)	0.22	1.66 (0.64 - 4.31)	0.3
	T3	**2.40 (1.12 - 5.13)**	**0.024**	**2.55 (1.03 - 6.30)**	**0.043**
Clinical model with NP (M3)	T2	1.54 (0.66 - 3.57)	0.32	1.49 (0.58 - 3.84)	0.41
	T3	1.76 (0.80 - 3.90)	0.16	1.87 (0.74 - 4.73)	0.19

In Siena cohort, LAV was calculated from the LAD using previously reported equation (1)

(1) Kobayashi, Masatake et al. “Machine Learning-Derived Echocardiographic Phenotypes Predict He art Failure Incidence in Asymptomatic Individuals.” JACC. Cardiovascular imaging vol. 15,2 (2022): 1 93-208. doi:10.1016/j.jcmg.2021.07.004

Figure 1S: **Example for converting 28 zone protocol B-line score to 8 zone protocol score.**



Number of B-lines in each LUS zone is in white font.

Method to count B-lines using highest count method from 28 zone LUS protocol

Zone 1: 4
\+ Zone 2: 6
\+ Zone 3: 8
\+ Zone 4: 3
\+ Zone 5: 4
\+ Zone 6: 4
\+ Zone 7: 4
\+ Zone 8: 3
Total B-lines in highest count method = 36

*Figure adapted with permission from Buessler, Aurélien et al. “Accuracy of Several Lung Ultrasound Methods for the Diagnosis of Acute Heart Failure in the ED: A Multicenter Prospective Study.” Chest vol. 157,1 (2020): 99-110.

4.1.1.1. Learning forward

We showed that LUS provides important prognostic information in patients with different LVEF subtypes and clinical settings. Although, prognostic capacity of B-lines was influenced by presence or absence of AF in the admission cohort, clinical characteristics did not influence the prognostic capacity of LUS in out-patients. A previous meta-analysis explored the prognostic capacity of LUS in different clinical settings [66], however, the present study was the first study to assess the impact of clinical variables (age, sex, LVEF and AF) on prognostic capacity of LUS. Another novel finding of this study is that addition of B-lines to clinical, MAGGIC and AHEAD score improved risk stratification in all clinical settings. A previous study had tested the prognostic utility of B-lines on top of HF risk score in a small number of HF patients at discharge (n = 123) however, the addition of B-lines was not associated with significant improvement in AUC curves when the risk scores contained natriuretic peptides levels [146]. Whereas, in our study, B-lines retained their prognostic capacity on top of clinical score consisting of NTproBNP and other variables suggesting its added value as an independent risk prognosticator. This difference in findings of the studies is possibly due to a larger sample size of this study.

An important observation in this study was that higher number of B-lines at admission was not significantly associated with outcomes in adjusted models. **This finding suggests that the effective decongestion may be a more important prognosticator than the initial severity of pulmonary congestion**. In addition, severe pulmonary congestion (highest tertile of B-line) was not associated with outcome in out-patient cohort after adjustment for natriuretic peptides possibly due to low statistical power in this low-risk subgroup.

Overall, the important takeaways of this study were 1) prognostic capacity of B-lines did not change depending on the clinical variables such as age, LVEF in different clinical settings. AF influenced the prognostic capacity in the admission cohort but not in discharge or out-patient cohort. This finding is important because AF impacts echocardiographic measurement

of E wave and e' wave which are crucial for prognostic evaluation in HF. Hence, B-line assessment can be particularly useful in patients with HF with AF. 2) B-lines are an independent risk predictor on top of natriuretic peptides in HF patients regardless of clinical setting. 3) Among in-patients, number of B-lines at discharge (indicating residual congestion) is more prognostically significant than B-lines at admission which probably reflects the importance of effective decongestion.

However, the important limitation of this study was that prognostic capacity of B-lines was analyzed in a cross-sectional manner, assessing prognostic capacity at admission and discharge separately. Moreover, clinical congestion assessment using other congestion scores or venous ultrasound were not available for the analysis.

4.2. Prognostic relevance of interplay of congestion sub-types

4.2.1. Poor in-hospital congestion improvement in acute heart failure patients classified according to left ventricular ejection fraction: prognostic implications.

The results of our first paper show that B-lines retain their prognostic capacity in HF subtypes and addition of B-lines improve risk stratification on top of MAGGIC and AHEAD score. This study demonstrated that pulmonary congestion is an independent risk factor for outcome in HF, particularly at discharge and in out-patient clinics. **However, to better understand the role of pulmonary congestion on patient outcomes, it would be crucial to evaluate its resolution as a prognostic factor.** The trend in the congestion changes, rather than the absolute congestion levels, may be the key to improving prognosis during the hospital stay. While a trend in B-line changes in a patient can give important information on patient profile and prognosis, the changes in clinical congestion score or natriuretic peptide levels may provide useful complementary information. This view, centered on the role of congestion changes, raises important questions which are as follows:

1) Whether limited or no improvement in any of the congestion parameters which encompass different dimensions of congestion – clinical congestion score, natriuretic peptides level and pulmonary congestion- are associated with worse prognosis?

2) In addition, changes in which of these congestion parameters may better stratify risk?

3) A patient may show improvement in a specific congestion parameter or in combination of parameters. In that case, limited improvement in which parameters or combination of parameters determines adverse outcomes?

4) Lastly, does LVEF categories impact the prognostic capacity of changes in congestion parameters?

In continuation of this work, we aimed to explore how in-hospital congestion changes from admission to discharge impact prognosis according to left ventricular ejection fraction

(LVEF) classification. Further, we studied whether limited improvement in clinical, biological and pulmonary congestion at discharge was related to outcome occurrence in HF subtypes. The primary outcome was a composite of all-cause mortality and/or HF re-hospitalization.

In this study, we assessed 244 patients with acute HF (mean age 80.3 ± 7.6 years) enrolled in two independent cohorts. To assess congestion severity, 1 point was attributed or each of the following signs: dyspnea at rest, pulmonary rales, third heart sound, jugular venous distention, peripheral oedema, and hepatomegaly. A simplified congestion score was based solely on pulmonary rales and peripheral oedema. Eight-zone LUS was conducted to assess total number of B-line counts at both admission and discharge.

We observed that limited/ minimal improvement in congestion parameters such as clinical congestion score (hazard ratio 2.33, 95% CI 1.51–3.61, $p = 0.0001$), NP levels (2.29, 95% CI 1.55–3.38, $p < 0.0001$), and the number of B-lines (6.44, 95% CI 4.19–9.89, $p < 0.001$) over the period of hospital stay was associated with increased risk of adverse outcomes.

A significant finding of this study was the observation of varying adverse event rates among patients who showed only no/limited improvement in diverse combinations of congestion parameters. Moreover, a limited improvement in B-line count even in presence of improvement of clinical and biological congestion was associated with higher adverse event rate.

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ORIGINAL PAPER

Poor in-hospital congestion improvement in acute heart failure patients classified according to left ventricular ejection fraction: prognostic implications

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ABSTRACT

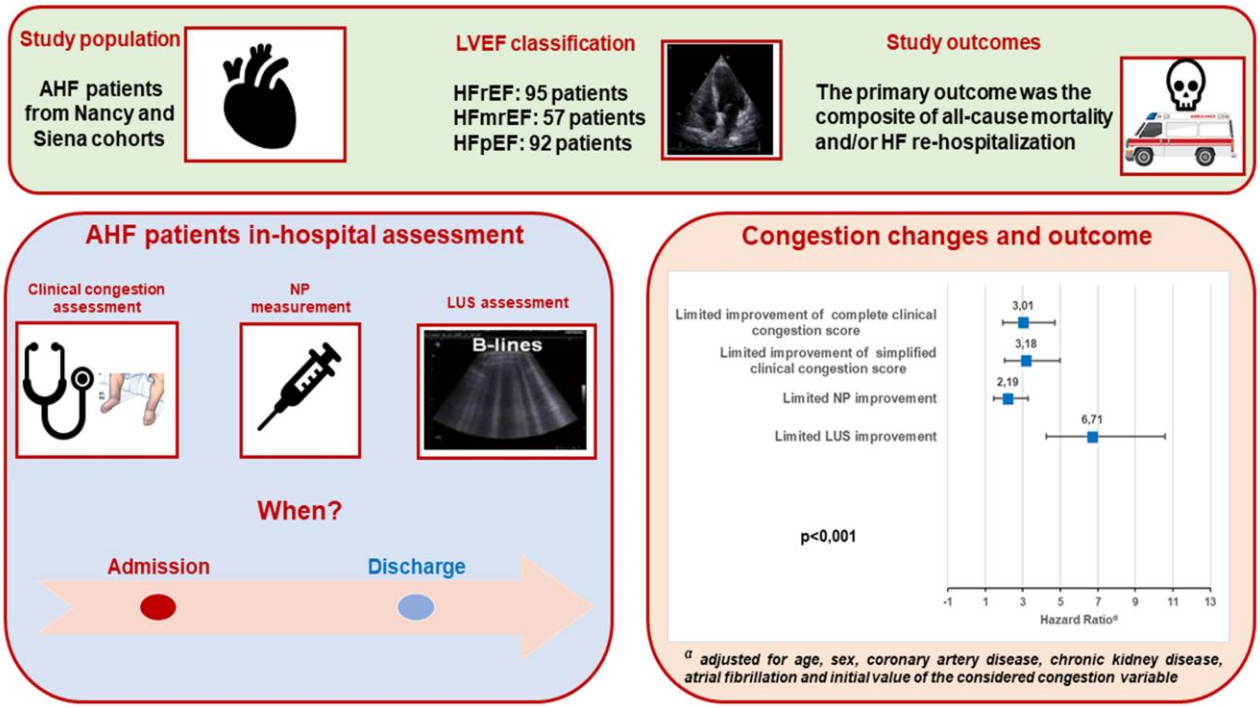
Background: Residual congestion in acute heart failure (AHF) is associated with poor prognosis. However, there is a lack of data on the prognostic value of changes in a combined assessment of in-hospital congestion. The present study sought to assess the association between in-hospital congestion changes and subsequent prognosis according to left ventricular ejection fraction (LVEF) classification.

Methods: Patients (N=244, 80.3±7.6 years, 50.8% male) admitted for acute HF in two European tertiary care centers underwent clinical assessment (congestion score included dyspnea at rest, rales, third heart sound, jugular venous distention, peripheral edema and hepatomegaly; simplified congestion score included rales and peripheral edema), echocardiography, lung ultrasound (LUS) and natriuretic peptides (NP) measurement at admission and discharge. The primary outcome was a composite of all-cause mortality and/or HF re-hospitalization.

Results: In the 244 considered patients (95 HF with reduced EF, 57 HF with mildly reduced EF and 92 HF with preserved EF), patients with limited improvement in clinical congestion score (hazard ratio 2.33, 95%CI 1.51 to 3.61, p=0.0001), NP levels (2.29, 95%CI 1.55 to 3.38, p<0.0001) and the number of B-lines (6.44, 95%CI 4.19 to 9.89, p<0.001) had a significantly higher risk of outcome compared to patients experiencing more sizeable decongestion. The same pattern of association was observed when adjusting for confounding factors. A limited improvement in clinical congestion score and in the number of B-lines was related to poor prognosis for all LVEF categories.

Conclusions: In AHF, the degree of congestion reduction assessed over the in-hospital stay period can stratify the subsequent event risk. Limited reduction in both clinical congestion and B-lines number are related to poor prognosis, irrespective of HF subtype.

Graphical abstract



INTRODUCTION

Despite the recent advances in heart failure (HF) therapies, approximately 20-30% of HF patients discharged from hospital are re-hospitalized within 30-90 days. [1,2] Acute heart failure (AHF) and acute decompensated heart failure (ADHF) are clinical syndromes characterized by progressive intravascular and interstitial fluid accumulation due to the increase in intracardiac filling pressures. Salt and water retention leads to both pulmonary and systemic congestion which are responsible for traditional symptoms and signs of decompensation such as dyspnea, hepato-renal congestion and peripheral edema. [3] Current scenario may vary according with HF typology, haemodynamic conditions, and disease journey (i.e. new onset vs worsening episode of chronic HF). In this framework, the timely and accurate detection of congestion and its resolution is one of the main objectives in AHF/ADHF management [3-5].

Various congestion assessment tools, such as clinical examination and chest X-ray, are typically employed for congestion detection in acute settings despite limited sensitivity [3-5]. Recently, lung ultrasound (LUS) has been endorsed in emergency departments to accurately diagnose and measure pulmonary congestion through the number of "B-lines" or lung "comets", which represent ultrasonic reverberations arising from extravascular lung water. [6-8] Indeed, several studies have demonstrated that the number of B-lines is a key prognostic marker in HF [9,10]. Additionally, residual pulmonary congestion at discharge assessed by LUS is reported to be associated with poor prognosis in HF patients. [9,10] Thus, in-hospital assessment of pulmonary congestion and the serial evaluation of its changes could become the "key" for clinicians to better monitor and treat AHF/ADHF patients.

The latest European Society of Cardiology (ESC) 2021 HF guidelines classify HF patients according to left ventricular ejection fraction (LVEF) status, namely heart failure with reduced ejection fraction (HFrEF, LVEF<40%), heart failure with mildly-reduced ejection fraction (HFmrEF, 40%≤ LVEF≤ 49%) and heart failure with preserved ejection fraction (HFpEF, LVEF≥50%). [11] Although several studies have evaluated the clinical characteristics and prognosis of HFrEF, HFmrEF and HFpEF

patients, there are very few studies which assessed the congestion burden in these specific patient subgroups.

The present study aimed to assess in-hospital changes/improvement in congestion and its association with prognosis in patients with HF according to LVEF class.

METHODS

Pooled Patient Population

This is a retrospective study combining datasets from two independent cohort studies which enrolled consecutive patients with AHF/ADHF.

The AHF-core study (admission and discharge): This is an ongoing cohort study which includes patients hospitalized for acute HF in the university hospitals of the Grand Est region of France. LUS was conducted within 72 hours of admission and before discharge.

The Siena cohort study (admission and discharge): The Siena cohort study included patients with ADHF and with sinus rhythm as previous shown. All patients underwent echocardiography within 12 hours after admission. Clinical, laboratory and LUS data were assessed both at admission and at discharge. [12]

All patients included in this study underwent clinical assessment, echocardiography, LUS and natriuretic peptides (NP) measurement during hospitalization (B-type natriuretic peptide [BNP] and N-terminal-pro-brain natriuretic peptide [NT-proBNP]).

Clinical congestion definition

Patients were evaluated by two physicians at admission and discharge to assess the degree of clinical congestion, with 1 point attributed for each of the following signs: dyspnea at rest, pulmonary rales, third heart sound, jugular venous distention, peripheral edema and hepatomegaly, all mentioned in the European Society of Cardiology scientific statement on "Assessing and grading congestion in acute heart failure" [5] and previously used by our team [12]. Simplified congestion score was based solely on pulmonary rales and peripheral edema.

Echocardiography

Echocardiography was performed at hospital admission. Left ventricular (LV) systolic and diastolic volumes and LVEF were determined using apical two- and four chamber views by the modified Simpson's biplane method. Left ventricular diastolic function was evaluated by pulsed Doppler transmitral flow velocity, including: early diastolic velocity (E wave), late diastolic velocity (A wave) and their ratio (E/A), deceleration time (DT) of E wave. Early diastolic myocardial velocity (e') was obtained by tissue Doppler imaging (TDI) on the basal septal and basal lateral walls at the level of the mitral annular site measuring five consecutive beats. The E/e' ratio was calculated from the mean of the two TDI measurements divided by the pulsed doppler E wave measured by placing the sample volume in the LV chamber between the two mitral leaflets during the diastolic phase. TAPSE was obtained by placing the M-mode cursor laterally to the tricuspid annulus. PASP was estimated by continuous Doppler at the tricuspid valve level as the sum of 4 * squared peak velocity of tricuspid regurgitation and the estimate of right atrial pressure based on inferior vena cava (IVC) diameter and collapsibility.

Lung Ultrasound (LUS)

Measurements were collected for each LUS zone. Eight LUS zones were analyzed for each patient. In the Siena cohort, the maximum number of vertical reverberation artifacts (B-lines) was counted in a single intercostal space for each zone and the sum of all eight zones was considered as the total number of B-lines. In the AHF-core cohort study, the number of B-lines was calculated from those

obtained with the twenty-eight-point method and converted to 8 zone counts as previously reported. [9]

Study Outcomes

The primary outcome was the composite of all-cause mortality and/or HF re-hospitalization. All patients were followed after discharge in each institution. Median follow-up duration in the AHF-core study was 90 (57 - 90) days and 156 (65 - 180) days in the Siena cohort study.

Statistical Analysis

Patients with heart failure were divided into three subgroups according to LVEF: HFrEF (LVEF≤40%), HFmrEF (40% <LVEF <50%) and HFpEF (LVEF≥50%).

Categorical data are presented as number and percentages, and normally and non-normally distributed continuous variables as mean and standard deviation (SD). Continuous variables were compared using ANOVA or Kruskal-Wallis tests as required, while chi-squared test or Fisher's exact test was used for categorical variables.

To assess the association between congestion changes and subsequent outcome, limited improvement in congestion was defined as an improvement in congestion below the median for each clinical, NP and LUS congestion variables. The thresholds used for limited improvement of each congestion variable were as follows: delta complete clinical congestion score ≥-2, delta simplified clinical congestion score ≥-1, delta NP Z score > 0, delta BNP > -411, delta B-lines >-10. Crude and adjusted Cox proportional hazard regression models were used to assess the relationship between congestion changes and clinical outcome during follow-up. In these models, interactions between LVEF category and the association of congestion variables with outcome were tested using interaction terms in survival models. Hazard ratios and their 95% confidence intervals are reported.

Results yielding a p value <0.05 were considered statistically significant. All analyses were performed using SAS version 9.4.6 (SAS Institute Inc., Cary, N.C., USA) and R version 3.6.1 (2019-07-05).

RESULTS

Baseline characteristics in the overall study population and according to LVEF class

A total of 244 patients were included (50.8% men, mean age 80.3±7.6 years). The proportion of ischemic heart disease (IHD), atrial fibrillation (AF) and chronic kidney disease (CKD) were 43.2%, 24.2% and 71.7 %, respectively. Mean admission clinical congestion score was 2.5±1.2 and mean admission BNP and mean NT-proBNP levels were 1354.1 ± 964.9 pg/mL and 16324.2 ± 20737.6 pg/mL, respectively. Mean LVEF was 41.6 ± 12.5 and mean B-lines at admission was 30.6 ± 9.2.

When sub-dividing the study population according to LVEF, there were 95 HFrEF patients, 57 HFmrEF patients and 92 HFpEF patients. Patients with HFrEF were more likely to be male (63.2% vs. 47.4% vs. 40.2% ; p=0.006) and had a higher prevalence of IHD (82.1% vs. 33.3% vs. 8.8% ; p<0.001) compared to HFmrEF and HFpEF patients. Moreover, patients with HFrEF demonstrated a significantly higher admission congestion score (3.0 ± 1.0 vs. 2.5 ± 1.2 vs. 2.0 ± 1.1 ; p<0.001) and admission BNP levels (1597.2 ± 1069.9 vs. 1324.3 ± 914.9 vs. 1121.1 ± 819.5 pg/mL ; p=0.001) relative to HFmrEF and HFpEF patients. Similarly, congestion score at discharge (complete) (p=0.0008) and BNP levels (p=0.0009) were significantly higher in HFrEF patients compared to HFmrEF and HFpEF patients. The number of B-lines at admission (p=0.14) and at discharge (p=0.11) did not differ significantly among the groups [Table 1].

In our cohort of 244 patients, 175 were identified with chronic kidney disease (CKD), defined by an estimated glomerular filtration rate (eGFR) of less than 60 ml/min/m^2. When examining the association between eGFR levels (categorized as eGFR<30 ml/min/m^2, 30 to less than 60 ml/min/m^2, and eGFR≥60 ml/min/m^2) and the number of B-lines at the time of admission, we found no statistically significant difference across the eGFR groups (p=0.26). This lack of significant

difference persisted between the number of B-lines at the time of discharge across the same eGFR categories (p=0.31), as illustrated in Supplementary Figure 1.

Association between limited improvement in congestion and outcome

Univariate Cox regression analysis showed that patients who experienced limited improvement in clinical congestion score (p=0.0001), NP levels (p<0.0001) and the number of B-lines (p<0.001) had a poor outcome. Multivariable Cox regression analysis confirmed a similar trend after adjustment for age, sex, coronary artery disease, CKD, AF and initial value of the considered congestion variable [Table 2].

When assessing the changes in clinical congestion, NP values and B-lines according to HFrEF, HFmrEF and HFpEF class, the limited improvement in clinical congestion score and in the number of B-lines were related to poor prognosis independently of LVEF classification. Among all variables, limited improvement in NP values during hospitalization was the only variable not significantly related to prognosis in HFpEF [Figure 1].

Interplay between limited improvement in clinical, imaging and biological congestion

In view of the above findings, the study population was divided into 7 subgroups according to the limited/no improvement in congestion variables using a Venn diagram. As showed in Figure 2, there were 35 patients with only limited/no improvement in simplified clinical congestion score, 30 patients with only limited/no improvement in the number of B-lines, 14 patients with only limited/no improvement in NP level, 20 patients with limited/no improvement in both simplified congestion score and NP level, 22 patients with limited/no improvement in both simplified congestion score and B-lines count, 33 patients with limited/no improvement in both B-lines count and NP level and 48 patients with limited/no improvement in simplified congestion score, B-lines count and NP level.

[Figure 2A] When analyzing the outcome occurrence rate among the 7 subgroups, limited improvement in clinical and/or NP level was associated with a poorer outcome whereas the limited improvement in B-lines count was associated with a high risk of adverse events occurrence irrespective of the presence of the two other components (17% vs. 62% vs. 36% vs. 10% vs. 59% vs. 88% vs. 73%; $p<0.001$) [Figure 2B].

Prognostic value of limited improvement in congestion according to LVEF class

The pattern of association between limited congestion improvement and outcome was similar in all LVEF subtypes [Figure 3].

DISCUSSION

Congestion plays a central role in HF progression and re-hospitalization and its assessment is a critical goal in HF management. [13,14] Several scores for clinical congestion evaluation and severity have been proposed although none have been extensively assessed to date and their related prognostic accuracy remains to be clarified. [15,16] Our study focused on the in-hospital evaluation of clinical and pulmonary congestion both at admission and at discharge. We demonstrate that the limited improvement in clinical congestion score, number of B-lines and natriuretic peptide levels from admission to discharge was related to poor outcome. In addition, the limited improvement in either clinical congestion score or B-lines count was associated with a higher risk of event regardless of LVEF class. A limited improvement in B-lines during hospital stay was associated with a numerically greater increase in event risk than limited improvement in clinical congestion score or natriuretic peptide levels. Hence, the limited improvement in B-lines count constituted the key congestion variable predicting worse prognosis in AHF.

To the best of our knowledge, this is the first study highlighting the prognostic role of in-hospital LUS changes in AHF/ADHF according to LVEF classification. AHF is a clinical syndrome characterized by fluid overload which manifests as clinical congestion. According to the traditional clinical classification, congestion status and perfusion state represent the main factors driving outcome in AHF patients. [3] In line with these observations, a recent analysis of the ESC-EORP-HFA Heart Failure Long-Term Registry demonstrated that, among 7865 AHF patients, "wet" patients had a worse prognosis; in addition, among the discharged patients, 30.9% had residual congestion, and had a higher 1-year mortality compared to patients discharged without congestion (28.0 vs. 18.5%). [17] Additionally, a recent study including 1000 patients showed that pulmonary congestion assessed through chest X-ray was related to poor prognosis, with a worsening of prognosis with increasing pulmonary congestion. [18] Given that LUS assessment appears to be superior to chest X-ray in detecting pulmonary congestion as shown in a head-to-head meta-analysis, accurate assessment of pulmonary congestion trajectories throughout the course of hospitalization is an invaluable tool for risk stratification. [19]

Moreover, our study showed that the number of B-lines at admission and at discharge did not significantly change across eGFR strata (categorized as eGFR<30 ml/min/m^2, 30 to less than 60 ml/min/m^2, and eGFR≥60 ml/min/m^2). These findings are different with respect to the available studies. Indeed, an analysis of 97 AHF patients performed by Pugliese et al. demonstrated that patients with CKD had higher percentage of patients with B-lines number>15 than patients without CKD. In this analysis, the concomitant presence of congestion and CKD was related to poor prognosis and also to inflammatory status. [20]

LUS is a rapid and reliable tool for assessing pulmonary congestion since it reflects real-time pulmonary congestion and pulmonary circulation status. Imanishi et al. confirmed the direct correlation between B-lines count and pulmonary capillary wedge pressure and thus the prognostic weight of residual pulmonary congestion in AHF. [21] Several studies assessing residual pulmonary congestion at discharge revealed the worse prognosis in patients with higher B-lines count.

[9,10,21,22] Our study confirms that a limited reduction in B-lines count (lesser than 9) was related to poor prognosis as well as the lack of improvement in clinical congestion.

Although all congestion signs are related to poor outcome in AHF, there is absence of concordance among the various congestion markers. [16] This lack of concordance likely results from the fact that every patient shows a different congestion phenotype which could be related to a different HF picture, etiology and LVEF classification. Despite the fact that peripheral congestion, intravascular congestion and pulmonary congestion are all aspects of the same pathophysiological process, they can all occur differently. Our findings are in agreement with a previous study including both HFrEF and HFpEF patients demonstrating the significant prognostic value of discharge B-lines count in all patients; a significant association between discharge B-lines and outcome was also separately confirmed in HFrEF and HFpEF. [12] Furthermore, a multicenter study including 125 patients with HFrEF and 83 patients with HFpEF, showed the residual pulmonary congestion, defined as the number of B-lines at discharge greater than 15, was related to poor prognosis independently from LVEF and from biohumoral and echocardiographic data. [23] Another report including approximately 1000 patients showed that pulmonary congestion detected through LUS (B-lines count>30) had a significant relationship with worse prognosis in HFpEF. [24] Conversely, Cogliati et al. did not find an association between outcome and B-lines, and only inferior vena cava (IVC) diameter at discharge was related to poor outcome. [25] Their findings suggested the prognostic weight of intravascular congestion in HFrEF patients who exhibited increased central venous pressure, a condition typically associated with systemic fluid accumulation and underlying right HF. Indeed, in HFpEF, prevalent pulmonary congestion assessed by LUS and its relationship with prognosis may result from fluid redistribution due to increased vascular resistance/stiffness, subsequently leading to reduced capacitance in the large veins as well as increased arterial resistance. [26]

Although various studies have analyzed congestion burden in both HFrEF and HFpEF, few studies have assessed such burden in HFmrEF. To this end, our study confirmed that the limited/no

improvement in clinical congestion and B-lines during hospitalization was related to poor prognosis independently from LVEF phenotype. Accordingly, HFmrEF patients with limited improvement in congestion demonstrated poor outcome, mainly related to poor B-lines improvement.

Thus, in-hospital assessment of congestion through clinical, laboratory examination and LUS at admission and after treatment could represent a valuable tool in recognizing high-risk profile patients. Patients with residual congestion were more prone to occurrence of adverse events, regardless of LVEF class. Our study further demonstrates the prognostic role of residual congestion as well as residual pulmonary congestion assessed by LUS. Congruent with these findings, Kang et al. showed that the combination of conventional congestion assessment and B-lines before discharge aided physicians in risk stratification of hospitalized HF patients. [27] Hence, limited congestion improvement during hospitalization may be related to unsuccessful or insufficient in-hospital treatment, diuretic resistance, or delay in prescribing newer HF therapies prior to discharge. A recent analysis of BLUSHED-AHF trial confirmed the better prognosis in patients with LUS reduction from emergency department to discharge; however, this benefit was significant and evident in patients without tachycardia, tachypnea, hypotension, hyponatremia, uremia, advanced age, or history of myocardial infarction at admission. [28] Indeed, in keeping with the latter, the ongoing IMP-OUTCOME trial will explore B-lines-guided intensive HF management in the post-discharge period in patients discharged with residual pulmonary congestion. [29] Therefore, the in-hospital monitoring of pulmonary congestion through LUS could be done together with echocardiography assessment evaluating PASP, inferior vena cava (IVC) and left ventricular filling pressures in order to better stratify patients with high risk of adverse events. This approach was applied by Ohman J et al. who demonstrated that E/e decrease within hospitalization was a significant congestion parameter able to evaluate pulmonary congestion resolution in relation to better prognosis in 60 AHF patients; however, pulmonary congestion resolution assessed by LUS, remained the only congestion variables related to good outcome at 6 months. [30] In this line, further studies are necessary to investigate the value of congestion monitoring and resolution to guide therapies in AHF patients.

LIMITATIONS

This study is a retrospective analysis of consecutive AHF patients, evaluated with a *post-hoc* research objective in mind. The methodology for recording B-lines differed between the two centers, utilizing either a 28-point or an 8-zone approach, which was later standardized. A notable limitation was the absence of echocardiographic assessments prior to discharge, which would have been valuable for a more detailed understanding of the changes in PASP, IVC size, and E/e' ratio throughout the hospital stay. Additionally, information on heart failure treatments during hospitalization was not comprehensively available for all patients, hindering the examination of the relationship between decongestive therapies and congestion patterns. Furthermore, data on heart failure triggers and comprehensive biomarker profiles during the acute phases were not accessible, thus precluding their analysis in relation to in-hospital congestion trends.

CONCLUSIONS

Limited improvement in clinical congestion, NP values and B-lines count is a strong predictor of poor prognosis irrespective of LVEF class. The prognostic value of limited improvement in B-lines appeared more stringent than that of other congestion parameters, making it a cardinal variable to monitor during hospital stay. Further studies should determine whether strategies intended to minimize discharge congestion (especially focusing on B-lines) can improve clinical outcome following discharge.

Conflict of interests: none to declare.

Funding resources: none.

Data availability: The study data are available for sharing after specific request to the corresponding author (Prof. Nicolas Girerd).

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Table 1: Characteristics of patients with heart failure overall and according to LVEF classification (HFpEF, HFmrEF and HFrEF) in the AHF-CORE and Siena cohorts

Characteristic	N	Overall (n=244)	HFrEF (LVEF <40%) (n=95)	HFmrEF (40<=LVEF <50%) (n=57)	HFpEF (LVEF >=50%) (n=92)	P-value
		Mean ± SD / n(%)	Mean ± SD / n(%)	Mean ± SD / n(%)	Mean ± SD / n(%)	
Age (years)	244	80.3 ± 7.6	80.9 ± 7.0	80.8 ± 7.5	79.5 ± 8.3	0.40
Men	244	124 (50.8 %)	60 (63.2 %)	27 (47.4 %)	37 (40.2 %)	0.006
Risk factors – n. (%)						
Previous HF	28	16 (57.1 %)	7 (58.3 %)	4 (57.1 %)	5 (55.6 %)	1.00
Ischemic heart disease (IHD)	243	105 (43.2 %)	78 (82.1 %)	19 (33.3 %)	8 (8.8 %)	< 0.0001
AF	244	59 (24.2 %)	24 (25.3 %)	15 (26.3 %)	20 (21.7 %)	0.77
CKD	244	175 (71.7 %)	66 (69.5 %)	43 (75.4 %)	66 (71.7 %)	0.73
Clinical examination and BNP						
Heart rate (bpm)	244	89.1 ± 9.6	88.9 ± 10.7	88.2 ± 10.8	89.8 ± 7.3	0.67
Systolic blood pressure (mmHg)	244	128.7 ± 18.1	116.6 ± 16.1	128.7 ± 12.8	141.3 ± 13.9	< 0.0001
Edema at admission	243	176 (72.4 %)	90 (94.7 %)	41 (71.9 %)	45 (49.5 %)	< 0.0001
Rales at admission	244	220 (90.5)	83 (87.4)	51 (89.5)	86 (94.5)	0.24
Admission congestion score (complete)	241	2.5 ± 1.2	3.0 ± 1.0	2.5 ± 1.2	2.0 ± 1.1	< 0.0001
0	241	1 (0.4 %)	0 (0.0 %)	1 (1.8 %)	0 (0.0 %)	
1	241	63 (26.1 %)	9 (9.7 %)	15 (26.3 %)	39 (42.9 %)	
2	241	57 (23.7 %)	22 (23.7 %)	15 (26.3 %)	20 (22.0 %)	<0.0001
3	241	53 (22.0 %)	23 (24.7 %)	9 (15.8 %)	21 (23.1 %)	
4	241	67 (27.8 %)	39 (41.9 %)	17 (29.8 %)	11 (12.1 %)	
Admission congestion score (simplified)	243	1.6 ± 0.5	1.8 ± 0.4	1.6 ± 0.5	1.4 ± 0.5	< 0.0001
0	243	2 (0.8 %)	0 (0.0 %)	1 (1.8 %)	1 (1.1 %)	
1	243	86 (35.4 %)	17 (17.9 %)	20 (35.1 %)	49 (53.8 %)	<0.0001
2	243	155 (63.8 %)	78 (82.1 %)	36 (63.2 %)	41 (45.1 %)	
Discharge congestion score (complete)	239	0.9 ± 1.2	1.2 ± 1.3	0.9 ± 1.2	0.7 ± 1.2	0.0008
0	239	125 (52.3 %)	34 (36.2 %)	31 (55.4 %)	60 (67.4 %)	
1	239	56 (23.4 %)	30 (31.9 %)	13 (23.2 %)	13 (14.6 %)	0.0008
2	239	21 (8.8 %)	14 (14.9 %)	5 (8.9 %)	2 (2.2 %)	

3	239	22 (9.2 %)	8 (8.5 %)	3 (5.4 %)	11 (12.4 %)	
4	239	15 (6.3 %)	8 (8.5 %)	4 (7.1 %)	3 (3.4 %)	
Discharge congestion score (simplified)	240	0.7 ± 0.8	0.8 ± 0.8	0.6 ± 0.8	0.5 ± 0.8	0.002
0	240	130 (54.2 %)	37 (38.9 %)	32 (57.1 %)	61 (68.5 %)	
1	240	64 (26.7 %)	36 (37.9 %)	15 (26.8 %)	13 (14.6 %)	0.0011
2	240	46 (19.2 %)	22 (23.2 %)	9 (16.1 %)	15 (16.9 %)	
BNP at admission	233	1354.1 ± 964.9	1597.2 ± 1069.9	1324.3 ± 914.9	1121.1 ± 819.5	0.001
BNP at discharge	230	887.3 ± 838.0	1117.1 ± 948.1	792.4 ± 715.0	701.6 ± 728.6	0.0009
NT-proBNP at admission	11	16324.2 ± 20737.6	33053.3 ± 26763.4	10632.3 ± 12298.6	3864.0 ± 1954.0	0.15
NT-proBNP at discharge	11	12315.6 ± 20317.0	25400.3 ± 31241.8	5618.3 ± 5803.8	4254.0 ± 4201.2	0.15
Echocardiography and LUS						
LVEF (%)	244	41.6 ± 12.5	28.4 ± 6.4	42.8 ± 2.7	54.5 ± 4.5	< 0.0001
B-lines count (highest scoring within a zone) at admission	244	30.6 ± 9.2	31.6 ± 9.4	30.1 ± 10.2	29.8 ± 8.4	0.14
B-lines count (highest scoring within a zone) at discharge	240	20.9 ± 9.2	22.4 ± 9.4	20.5 ± 9.7	19.6 ± 8.6	0.11

Abbreviations: LVEF: Left Ventricular Ejection Fraction, HFrEF: Heart Failure with reduced Ejection Fraction, HFmrEF: Heart Failure with mildly reduced Ejection Fraction, HFpEF: Heart Failure with preserved Ejection Fraction, BNP: B-type natriuretic peptide, NT-proBNP: N-terminal-pro-brain natriuretic peptide, LUS: Lung Ultrasound

Complete congestion score was based on 1 point being attributed for each of the following signs: dyspnea at rest, pulmonary rales, third heart sound, jugular venous distention, peripheral edema and hepatomegaly. Simplified congestion score was based solely on pulmonary rales and peripheral edema.

Table 2: Association between limited improvement in congestion and the composite of death or re-hospitalization for heart failure - Overall.

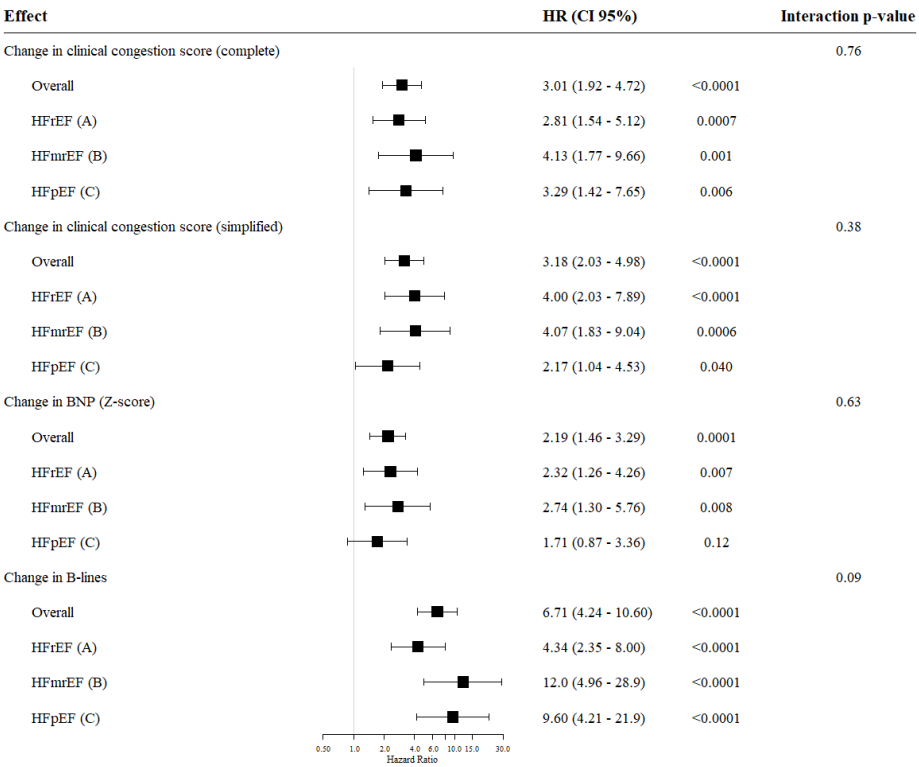
		Univariable		Adjusted [1]	
	n/N	HR (95%CI)	P-value	HR (95%CI)	P-value
Limited clinical congestion improvement					
Based on complete clinical congestion score	185/237	2.33 (1.51 - 3.61)	0.0001	3.01 (1.92 - 4.72)	<0.0001
Based on simplified clinical congestion score	186/240	2.04 (1.36 - 3.07)	0.0006	3.18 (2.03 - 4.98)	<0.0001
Limited NP improvement		2.17 (1.49 - 3.17)	<0.0001	2.19 (1.46 - 3.29)	0.0001
Based on median NP Z score change	125/241				
Relative reduction from admission >30%	62/241	0.93 (0.62 - 1.41)	0.74	1.14 (0.75 - 1.73)	0.55
Based on median BNP change	115/230	2.29 (1.55 - 3.38)	<0.0001	2.59 (1.69 - 3.99)	<0.0001
Limited LUS improvement					
Based on median B-lines change	116/240	6.44 (4.19 - 9.89)	<0.0001	6.71 (4.24 - 10.60)	<0.0001

[1] adjusted for age, sex, coronary artery disease, chronic kidney disease, atrial fibrillation and initial value of the considered congestion variable

Complete congestion score was based on dyspnea at rest, pulmonary rales, third heart sound, jugular venous distention, peripheral edema and hepatomegaly. Simplified congestion score was based solely on pulmonary rales and peripheral edema.

The thresholds used for each congestion variable are as follows: delta complete clinical congestion score ≥ -2, delta simplified clinical congestion score ≥ -1, delta NP Z score > 0, delta BNP > -411, delta B-lines > -10.

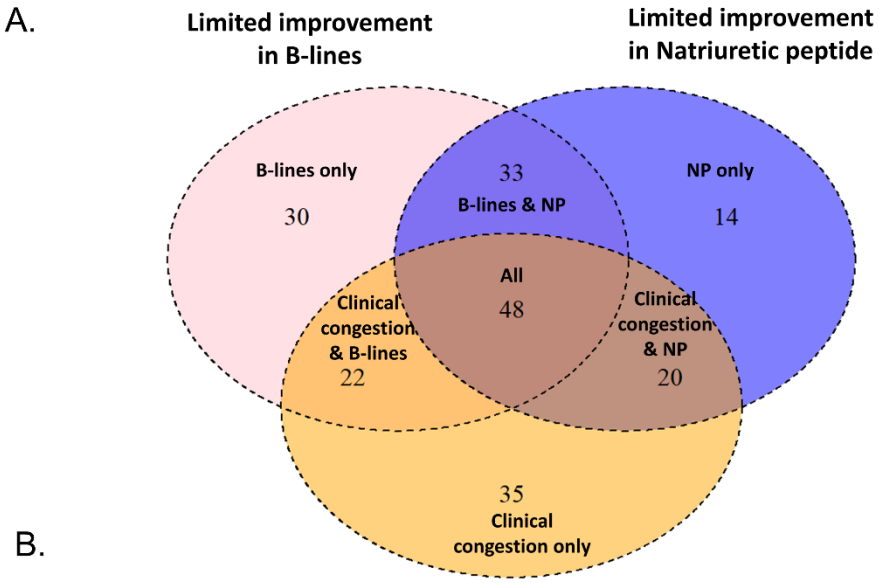
Figure 1: Association between limited improvement in congestion and the composite of death or rehospitalization for heart failure according to HF subtype.

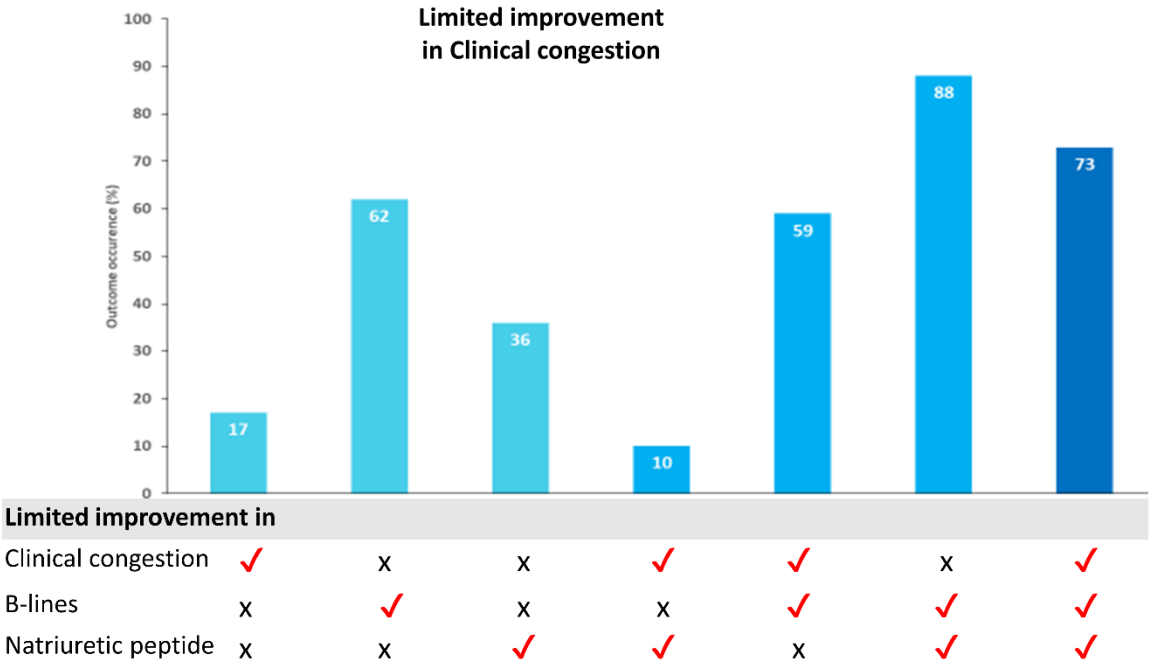


Legend: Models are adjusted for age, sex, coronary artery disease, chronic kidney disease, atrial fibrillation and initial value of the considered parameter.

The thresholds used for each congestion variable are as follows: delta complete clinical congestion score ≥ -2, delta simplified clinical congestion score ≥ -1, delta NP Z score > 0, delta BNP > -411, delta B-lines > -10.

Figure 2: Venn diagram according to limited/no congestion improvement in congestion parameters (A); Outcome occurrence rate according to limited/no improvement congestion groups (Chi-square test, p<0.001) (B).

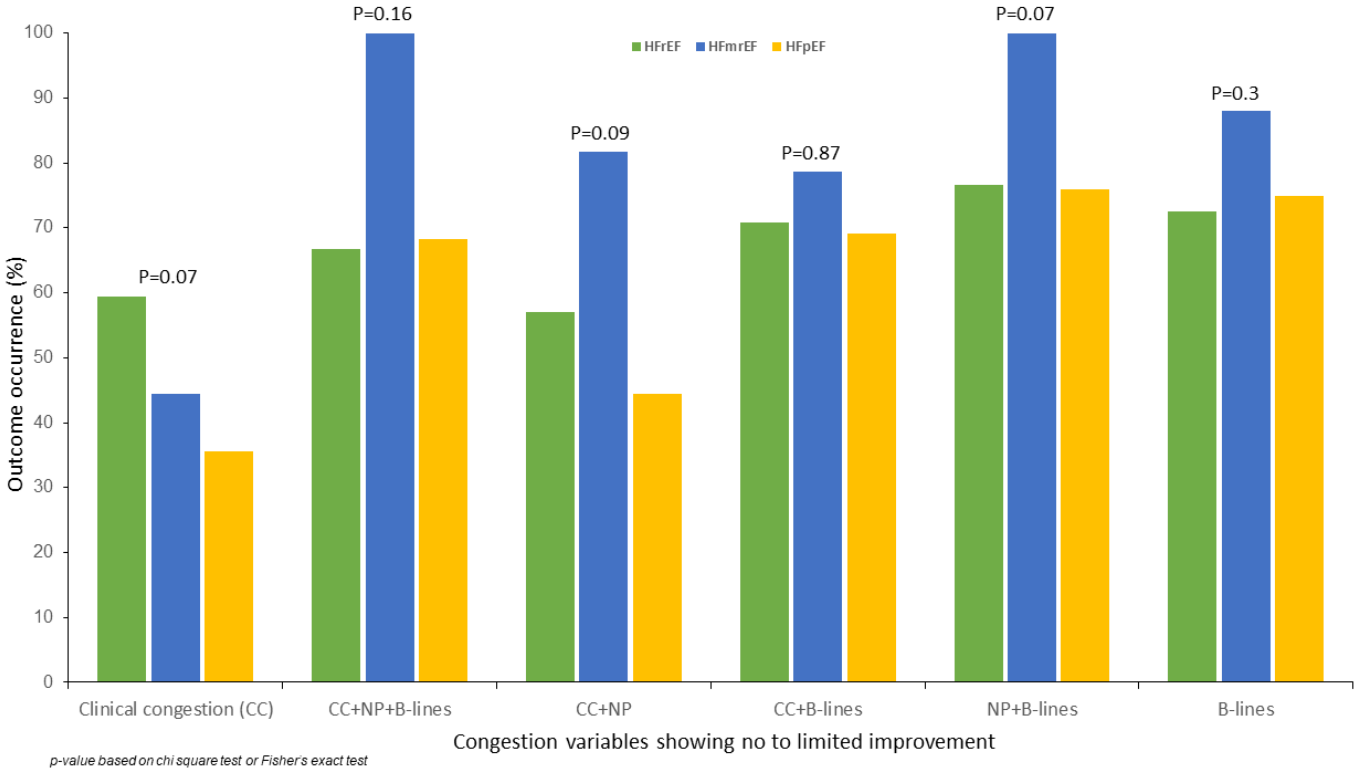




Limited improvement in							
Clinical congestion	✓	x	x	✓	✓	x	✓
B-lines	x	✓	x	x	✓	✓	✓
Natriuretic peptide	x	x	✓	✓	x	✓	✓

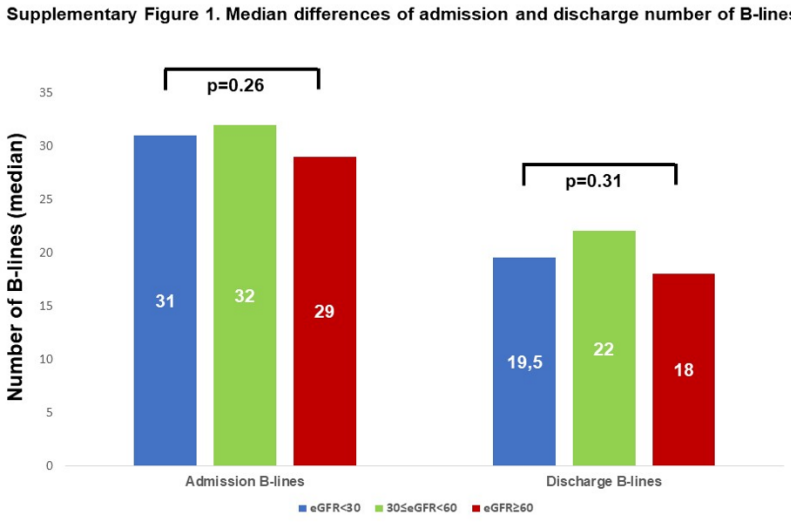
Legend: (✓) indicates the limited to no/ congestion improvement in the congestion parameter.
(x) indicates improvement in the congestion variable.

Figure 3: **Percentage of patients with outcome occurrence according to limited/no improvement congestion variables and HF subtype**



Supplementary data

Supplementary Figure 1: Median differences of admission and discharge number of B-LINES according to eGFR strata.



4.2.1.1. Learning forward

In this study we showed that poor in-hospital changes in clinical congestion, NP levels and B-lines were associated with worse prognosis. **Moreover, the B-lines were associated with numerically higher risk compared to clinical congestion scores in different HF profiles (HR: 6.71 (4.24 – 10.60) vs 3.01 (1.92 – 4.72)).** Adverse event rate was higher if B-lines did not show improvement even though an improvement in clinical and NP levels was observed suggesting that prognosis may vary depending on the improvement of specific congestion parameters. In this analysis, we also assessed if number of B-lines varied according to renal function status in HF. At both admission and discharge, number of B-lines was similar in patients with different renal function status (categorized as eGFR<30 ml/min/m^2, 30 to less than 60 ml/min/m^2, and eGFR≥60 ml/min/m^2). A recent study showed similar finding that evolution of B-lines during hospital stay was independent of creatinine levels but changed rapidly after diuretics [147].

While this study assessed imaging modality (LUS) to better quantify pulmonary congestion in HF patients, we could not assess the systemic venous congestion. This is relevant as ultrasound measures of pulmonary and systemic venous congestion have a moderate correlation at best implying that they reflect different dimensions of congestion. Moreover, different degrees of pulmonary and systemic congestion interaction may influence prognosis. This highlights important research gap in context of ultrasound measures of congestion which are as follows:

1) How frequent is the co-existence of pulmonary and systemic congestion in patients with HF?
2) Isolated severe pulmonary congestion or venous congestion portends similar or different risk?
3) Whether patient stratification based on degree of pulmonary and systemic congestion interplay better predicts the prognosis than the evaluation of pulmonary and systemic congestion (without interplay).

Hence, in the next study paper we evaluated the prognostic value of the relation between pulmonary and systemic congestion in HF.

4.2.2. Systemic and pulmonary congestion by ultrasound and prognosis in heart failure: a pooled cohort analysis

Assessment of B-lines by LUS improves the accuracy of pulmonary congestion management [16,67]. However, levels of pulmonary congestion do not always correlate with systemic intravascular congestion. A comprehensive ultrasound examination of both pulmonary and systemic congestion could provide a clearer picture of congestion patterns. Moreover, **how the co-existence of pulmonary and systemic congestion is associated with prognosis in patients with HF is not clear.**

We examined 835 patients (500 in-patients and 335 out-patients) enrolled in five different European cohorts. In this analysis we grouped the patients into six groups according to tercile of B-lines and IVC congestion status among in-patients and out-patients separately. The reference group was patients with mild pulmonary congestion (lowest tercile of B-lines, <16 for in-patients, <3 for out-patients) and un-dilated IVC (<21mm).

We found that dilated IVC was associated with a greater risk in both in-patients and out-patients when pulmonary congestion was more than mild. In addition, out-patients with highest degree of pulmonary oedema without IVC dilatation were also at increased risk of adverse outcome. **This study concluded that a combination of both pulmonary and systemic congestion is associated with worse prognosis whereas isolated systemic and pulmonary congestion is less predictive of poorer outcomes.**

Our study provides real-world data substantiating the conceptually established pulmonary and systemic categorization of congestion into tissue /vascular and pulmonary /systemic components .

Systemic and pulmonary congestion by ultrasound and prognosis in heart failure: a pooled cohort analysis

Short Title: LUS, IVC and prognosis in heart failure.

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Introduction

Renal retention of water and salt, or congestion, is an important feature of heart failure (HF) and a leading cause of hospitalization and mortality (1, 2). In a failing heart, the filling pressure required to maintain cardiac output increases: in the case of left sided HF, interstitial and eventually alveolar pulmonary edema may result. Compared with auscultatory findings, such as respiratory crackles, or a chest-x-ray, lung ultrasound (LUS) has greater sensitivity in detecting extravascular fluid accumulation in the lungs (3). For this reason, LUS is recommended to confirm a diagnosis of HF, particularly when natriuretic peptides are not routinely available, and its use is increasing in research and clinical practice

to facilitate HF management (4-6). Persistently elevated filling pressure causes increased pulmonary vascular resistance with raised post and pre-pulmonary capillary pressure. These pressure changes can ultimately lead to right heart dysfunction and systemic venous congestion. Inferior vena cava (IVC) diameter can be measured by ultrasound to estimate right atrial pressure and detect intravascular volume expansion.

Assessment of B-lines and IVC by ultrasound is safe, provides a rapid, non-invasive real-time estimate of pulmonary and systemic venous congestion, and identifies patients with either acute or chronic HF at greater risk of cardiovascular events, regardless of their left ventricular ejection fraction (LVEF) (5, 7-11).

The correlations between ultrasound measures of pulmonary and systemic venous congestion are modest at best, suggesting that ultrasound examination of congestion might be misleading if not performed comprehensively (12, 13). Moreover, the prognostic value of LUS and IVC diameter in patients with HF has been studied separately (14, 15). Understanding the interplay between different ultrasound features of congestion might provide a more complete picture and guide an optimal management (16, 17). Hence, in this study we assessed how the co-existence of pulmonary and systemic congestion associates with prognosis in patients with HF.

Methods

We performed a pooled analysis of independent European cohort studies that enrolled in-patients or out-patients with HF. For this analysis, we had data on IVC and LUS for the following cohorts:

In-patients:

1) AHF-core. This is an ongoing, prospective, multi-center, cohort study (NCT03327532) conducted in the Grand-Est region of France enrolling patients hospitalized with HF. Patients are assessed for clinical and laboratory data at admission; echocardiography and LUS are conducted within 72 hours.

2) Nancy cohort study. This is a retrospective study that included in-patients with acute HF hospitalized at "Département de Cardiologie Medicale", Nancy, France, between April 2014 and August 2015 (18). Echocardiography and LUS were performed within 72 hours of admission. Collection of clinical details for research purposes was approved by the Commission Nationale Informatique et Libertés (CNIL), under French law for single-center usual care observational studies.

3) Pisa cohort study. This was a prospective study that enrolled consenting patients admitted with acute HF in a cardiology department of a tertiary hospital in Pisa, Italy, between October 2004 and October 2008. Echocardiography and LUS were performed within 12 hours of ward admission (19).

4) Siena cohort study. In this study, consenting patients admitted to the cardiology unit in Siena, Italy, with a clinical diagnosis of a new-onset or worsening HF and in sinus rhythm between 2014 and 2018, were enrolled within 12 hours of hospital admission (20).

Out-patients:

1) Hull LifeLab includes a cohort of consecutive consenting patients with a prior clinical diagnosis of HF attending a routine specialist follow-up visit between April 2016 and March 2017 (12) in Hull, UK. Patients had clinical, echocardiography, laboratory, and lung ultrasound assessment during the same visit.

Main exclusion criteria in the cohorts were: (i) Inability to provide informed consent; (ii) Important lung disease potentially affecting LUS findings, such as pulmonary fibrosis, previous pneumectomy or lobectomy, pulmonary cancer or metastases; (iii) Other severe conditions hampering image acquisition. Each cohort study was conducted in accordance with the Declaration of Helsinki and was approved by the respective Ethics Committees.

Ultrasound

Echocardiograms were performed by experienced operators using different models of ultrasound machines and analyzed locally. Left ventricular ejection fraction (LVEF) was measured using Simpson's biplane method and tricuspid annular plane systolic excursion (TAPSE) was used to assess right ventricular systolic function.

For in-patients, the eight-zone LUS scanning protocol was adopted in Siena Cohort. The maximum number of B-lines counted in each zone was summed to calculate the total number of B-lines. For other cohorts of in-patients, the 28-zones scanning protocol was used: we derived the corresponding 8-zone total number of B-lines as previously reported (21). Pulmonary congestion was categorized using terciles of B-lines as mild (T1 <16), moderate (T2 ≥16 to 29), or severe (T3 >30). A similar grading of severity of congestion based on LUS has been published previously (22).

For the outpatient cohort, the 28-zone scanning protocol was employed. Total number of B-lines for the outpatient department (OPD) was also classified into tertiles: T1 (0 to <3), T2 (≥3 to <12), and T3 (≥12).

The IVC diameter was measured in the subcostal view. The IVC was defined as dilated if the diameter was >21 mm (23). In both in-patients and out-patients, systemic congestion was categorized in two groups as no systemic congestion (<21 mm) and overt systemic congestion (≥ 21mm).

Study groups

Thereafter, we divided the patients into six groups according to tercile of B-lines and IVC congestion status to explore the relations between IVC diameter, number of B-lines and outcomes, among in-patients and out-patients separately:

- Mild pulmonary congestion, no systemic congestion: B-line T1 & IVC < 21 mm (reference group)

- Mild pulmonary congestion, overt systemic congestion: B-line T1 & IVC ≥ 21mm
- Moderate pulmonary congestion, no systemic congestion: B-line T2 & IVC < 21 mm
- Moderate pulmonary congestion, overt systemic congestion: B-line T2 & IVC ≥ 21mm
- Severe pulmonary congestion, no systemic congestion: B-line T3 & IVC < 21 mm
- Severe pulmonary congestion, overt systemic congestion: B-line T3 & IVC ≥ 21mm

Study Outcomes

The primary outcome was a composite of hospitalization for HF and all-cause death. Outcome data were collected from patients, by phone interviews or during clinical appointments, or their family doctors, from review of the patient's hospital record or extracted from the electronic systems of the hospital where available (i.e.: Hull LifeLab).

Statistical Analysis

Categorical data were presented as number and percentage; continuous variables as median (25th-75th percentile). Continuous variables were compared using Kruskal-Wallis tests, while chi-squared tests or Exact-Fisher's were used for categorical variables. Correlation between total number of B-lines and IVC diameter was assessed using Pearson's coefficient. To assess the association between systemic and pulmonary congestion with clinical outcomes, we used crude and adjusted Cox proportional hazard regression models. While recognizing the limitations of categorizing continuous variables, we analyzed IVC diameter and B-lines both as continuous and categorical variables in separate models. Models were adjusted by selecting, a priori, a limited number of variables largely available in the dataset and known to be associated with poorer outcomes (age, estimated glomerular filtration rate, left ventricular ejection fraction); hazard ratios (95% confidence intervals) are reported. To account for variation across admission cohorts, the Cox-proportional hazard models were stratified by study groups. Kaplan–Meier curves with the log-rank statistic were used to illustrate outcome. All analyses were performed using SAS

version 9.4.6 (SAS Institute Inc., Cary, N.C., USA) and R version 4.0.2 (R Core Team (2020)). A two-sided P-value < 0.05 was considered statistically significant.

Results

Demographic and clinical characteristics of the study population

Of the 835 patients with HF (mean age (75 (±11) years, 60% men), 500 (60%) were in-patients. Patient characteristics for in-patients and out-patients are shown in table 1 and 2, according to their congestion status by ultrasound. Only a few in-patients had mild pulmonary congestion and overt systemic congestion (n=36, 7%) or severe pulmonary congestion and no systemic congestion (n=33, 7%). Compared to in-patients with mild pulmonary congestion and no systemic congestion (n= 124, 25%), those with greater congestion had more severe symptoms and clinical signs of congestion, and higher natriuretic peptides. Patients with both overt systemic and severe pulmonary congestion (n=138, 28%) were the oldest, the most congested, with the highest natriuretic peptide levels. Patients with more congestion had worse right ventricular function as measured by TAPSE, but LVEF was similar amongst groups.

Amongst out-patients, those (n=21, 6%) with mild pulmonary congestion and overt systemic congestion had higher natriuretic peptides compared to those with mild or moderate pulmonary congestion and no systemic congestion. Those with overt systemic and severe pulmonary congestion had the highest NT-proBNP and were more likely to be treated with loop diuretics than others. Compared to in-patients, a more detailed characterization by ultrasound was available for out-patients: those with systemic and severe pulmonary congestion had the greatest left atrial size, worst right ventricular function and were more likely to have moderate/severe mitral and tricuspid regurgitation than others, but there was no difference in LVEF amongst groups (Table 2).

B-lines and IVC diameter were modestly correlated in both admission (Pearson correlation coefficient: 0.42, 95%CI: 0.34- 0.49) and outpatient cohorts (0.37, 95% CI: 0.27- 0.46; p<0.0001 for both).

Outcomes

Over a follow-up of 180 days, 163 (33%) in-patients had died or were hospitalized with HF. Among out-patients, 125 (37%) had died or were hospitalized with HF over a median follow-up of 600 (366-747) days.

In crude analysis, both B-lines and IVC as continuous variables were associated with outcome in in-patient (HR= 1.01 (1.00 - 1.03), p= 0.023 and 1.10 (1.07 - 1.13), p<0.0001 respectively) and out-patient cohort (HR = 1.02 (1.01 - 1.02), p<0.0001 and 1.08 (1.06 - 1.10), p<0.0001 respectively). We identified a significant interaction between these 2 variables in the admission cohort (p-interaction = 0.02) but not in the outpatient cohort (p-interaction =0.4). Among in-patients, only increasing IVC was significantly associated with worse outcome after adjustment, whereas in out-patients, both increasing B-lines and IVC were associated with worse outcome (supplementary table 1).

Among in-patients, compared to patients with mild pulmonary congestion and no systemic congestion, those with isolated moderate or severe pulmonary congestion did not have an increased risk of events in the univariable and adjusted models. The risk increased only when moderate pulmonary congestion was concurrent with overt systemic congestion, and was the highest when there was severe pulmonary congestion and overt systemic congestion (HR: 2.32 (1.32- 4.10), p =0.004) (Figure 1).

In outpatients, compared to patients with mild pulmonary congestion and no systemic congestion, the presence of moderate pulmonary congestion and no systemic congestion was not associated with poorer outcomes, whereas patients with severe pulmonary congestion and no systemic congestion had an greater risk (HR: 2.34 (1.11- 4.91), p = 0.025). The combination of moderate or severe pulmonary congestion with systemic congestion was also associated with a significantly higher risk of poor prognosis (HR: 2.62 (1.24- 5.52), p = 0.012) and HR: 4.19 (2.24 – 7.83), p <0.0001, respectively) (Figure 2).

Discussion

Systemic and pulmonary congestion are associated with poorer outcomes in HF and are a key target of treatment. Using pooled data from multiple European cohorts, we expand previous findings, as we have found that concomitant pulmonary and systemic congestion as measured by higher count of B-lines and dilated IVC diameter, in both in-patients and out-patients, is associated with higher risk of hospitalization for HF and death. We also found that isolated systemic and pulmonary congestion is less predictive of poorer outcomes in these populations.

Identification and management of congestion in patients with HF are two difficult tasks for healthcare professionals. Ultrasound, given its versatility and wide availability, is not only the most used test to diagnose and quantify severity of cardiac dysfunction in patients with suspected or overt HF but is gaining popularity to detect congestion in different organs and tissues and identify patients at greater risk with more precision (24). When clinical congestion is severe, ultrasound signs of congestion, including a high number of B-lines and a dilated inferior vena cava, are usually present and concordant; in that context, priority should be given to the identification of the reasons leading to decompensation and to treating a very dangerous situation.

In addition, ultrasound can be a valuable ally for the clinician when there is a high clinical suspicion of worsening congestion—such as when a patient reports more severe breathlessness on exertion—but clinical signs of congestion, like lung crackles or elevated jugular venous pressure, are subtle. Previous studies have demonstrated that clinical examination often underestimates the likelihood of pulmonary or systemic congestion, or – importantly – of HF (25-27). In our study, the total number of B-lines and IVC diameter correlated only modestly in both in-patients and out-patients. Our findings suggest that assessment of congestion by ultrasound in patients with HF should be comprehensive and include evaluation of both to improve risk stratification.

Congestion is now classified as tissue and hemodynamic as well as pulmonary vs systemic (16, 17). Although this four-class categorization of congestion is conceptually well established, its real-world clinical value remains largely unexplored. Our results show that the combination of hemodynamic systemic congestion (as measured by IVC) and tissue pulmonary congestion (B-lines) are synergistically associated with outcome. Distinct pathophysiological mechanisms contribute to the development of predominant pulmonary and/or systemic congestion (17). Systemic congestion may arise from the combination of neurohormonal and hemodynamic factors which lead to substantial salt and water retention and plasma volume expansion. IVC dilatation is related to right atrial pressure but can also to some extent be favored by right ventricular dysfunction and more advanced HF. Hence, systemic congestion may be more difficult to treat compared to pulmonary congestion. In contrast, pulmonary congestion is caused by accumulation of interstitial fluid due to imbalance of hydrostatic and oncotic pressure, often due to a sudden increase in left ventricular end diastolic pressure and reduced pulmonary vein capacitance. Moreover, improvement in pulmonary congestion during hospitalization does not necessarily correlate with improvement in clinical signs of congestion or natriuretic peptide levels (28).

Given the differences in pathophysiological mechanisms, it has been suggested that diuretics may be more suited for treating systemic congestion while vasodilator therapies might be effective in treating patients with acute onset of high blood pressure and suddenly increased cardiac filling pressure (29, 30). Thus, a comprehensive congestion assessment may also improve patient management (31).

HF guidelines (4) do not recommend echocardiography if clinical deterioration is not suspected. Instead, symptoms, signs and daily body weight, should be monitored despite their low specificity and sensitivity in identifying worsening congestion. The development of lightweight, portable, hand-held ultrasound devices and affordable transducers that can be easily connected to any tablet or mobile phone has opened a new world of possibilities to anticipate diagnosis and individualize management of congestion. Lung B-lines and IVC diameter can be assessed rapidly in any setting, including in primary care, by medics and other healthcare providers (32, 33), complementing clinical history and examination, to facilitate the identification of those who might require reassurance, intensification of diuretic treatment or referral for specialist care. The consensus statement from EACVI (European Association of Cardiovascular Imaging) has recently recommended that LUS should complement echocardiography systematically in the setting of HF (5). In this context, our findings are very important. However, a standardized method for assessing B-lines is required to ensure consistent application of LUS and expand its diagnostic relevance and awareness.

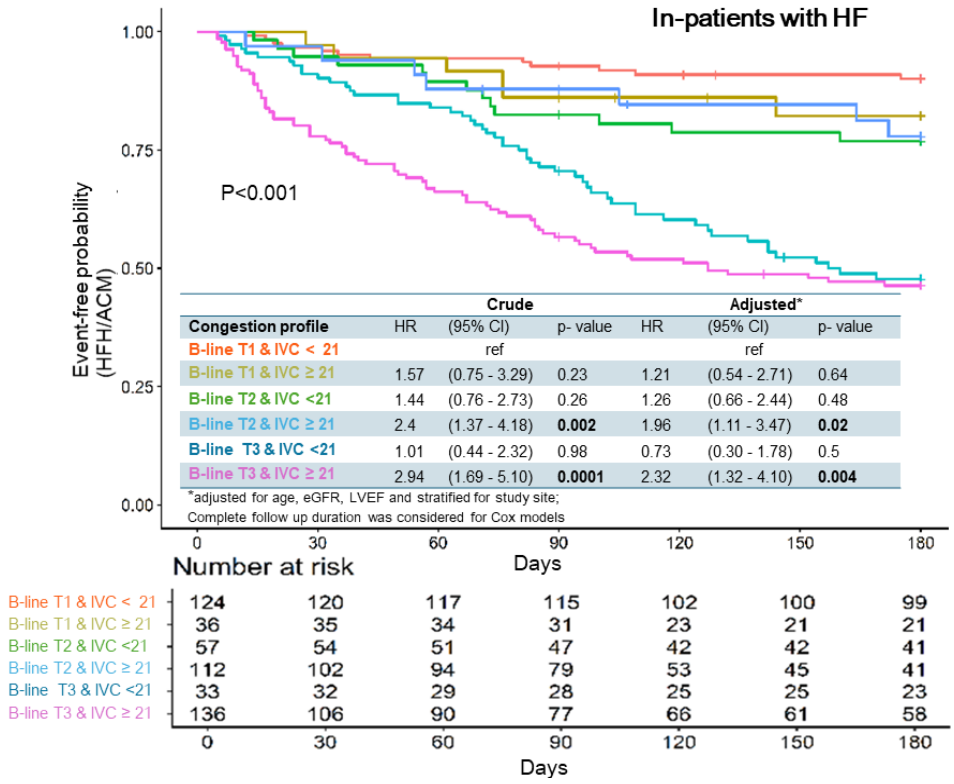
There are several limitations to be acknowledged. Only patients who had both IVC and B-lines assessed by ultrasound were included in this analysis, and selection bias might have occurred. Differences in diagnostic criteria for acute HF amongst individual cohorts might have led to the inclusion of a heterogeneous, but nevertheless, representative, population of patients. There was no agreement on how B-lines should be assessed when initial studies were performed (5), which is reflected in the different

protocols used in the various cohorts included in this study. Most of the in-patients enrolled in this study would have received loop diuretics, in different doses and via different routes of administration, prior to being assessed by ultrasound that might have influenced the number of B-line and IVC diameter differently. Moreover, concomitant diseases, such as respiratory infection or pulmonary hypertension, could have also impacted LUS findings. The VExUS approach combining hepatic, portal and intra-renal vein ultrasound has been proposed to stratify risk in HF when the IVC is dilated (34); however, other ultrasound measures of intravascular or renal congestion were not performed in this study (35, 36). There is no agreement in what constitutes a HF hospitalization (37), and heterogenicity in adjudication procedures might have introduced additional biases.

Conclusions

Amongst patients with HF, the combination of both pulmonary and systemic congestion is associated with a worse prognosis than either sign of congestion alone. Whether a more systematic assessment of pulmonary and systemic congestion by ultrasound can improve risk stratification, guide management, and improve outcomes in routine practice in those with HF requires more consideration.

Figures



	Crude			Adjusted*		
Congestion profile	HR	(95% CI)	p- value	HR	(95% CI)	p- value
B-line T1 & IVC < 21	ref			ref		
B-line T1 & IVC ≥ 21	1.57	(0.75 - 3.29)	0.23	1.21	(0.54 - 2.71)	0.64
B-line T2 & IVC <21	1.44	(0.76 - 2.73)	0.26	1.26	(0.66 - 2.44)	0.48
B-line T2 & IVC ≥ 21	2.4	(1.37 - 4.18)	**0.002**	1.96	(1.11 - 3.47)	**0.02**
B-line T3 & IVC <21	1.01	(0.44 - 2.32)	0.98	0.73	(0.30 - 1.78)	0.5
B-line T3 & IVC ≥ 21	2.94	(1.69 - 5.10)	**0.0001**	2.32	(1.32 - 4.10)	**0.004**

*adjusted for age, eGFR, LVEF and stratified for study site;
Complete follow up duration was considered for Cox models

Figure 1: Kaplan-Meier curves for the primary composite endpoint (heart failure hospitalization (HFH) or all-cause mortality (ACM)) in in-patients with HF with or without congestion by ultrasound. Compared to those without systemic (IVC<21 mm) and with mild pulmonary congestion (1st tercile (T1) of lung B-lines, in orange), those with isolated pulmonary congestion had a similar risk of events. The risk was higher for those with both moderate and severe pulmonary congestion concurrent with systemic congestion. Crude and adjusted risk estimates for different subgroups of patients are shown, according to the presence or absence of congestion.

* The hazard ratios for admission cohort are presented for complete follow-up. Over a median follow up of 180 days (90- 414), 193 patients had HFH or ACM.

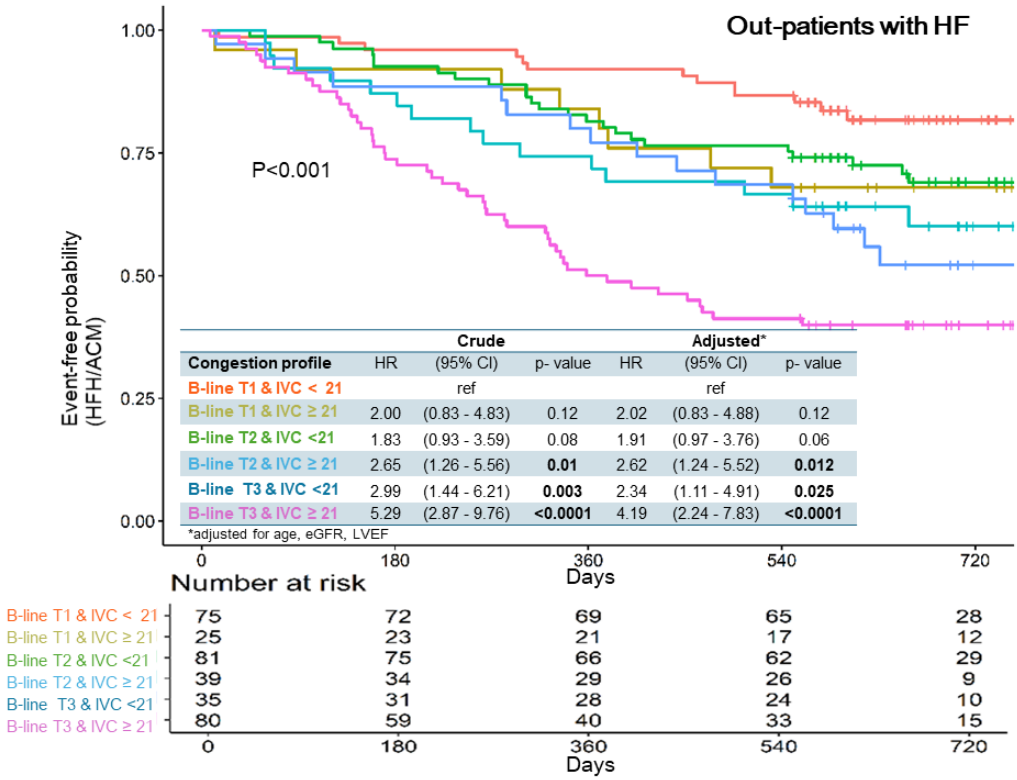


Figure 2: Kaplan-Meier curves for the primary composite endpoint (heart failure hospitalization (HFH) or all-cause mortality (ACM)) in out-patients with HF with or without congestion by ultrasound. Compared to those without systemic (IVC<21 mm) and mild pulmonary congestion (1st tercile (T1) of lung B-lines, in orange), those with severe isolated pulmonary congestion had a worse outcome. Those with severe pulmonary congestion concurrent with systemic congestion had the greatest risk. Crude and adjusted risk estimates for different subgroups of patients are shown, according to the presence or absence of congestion.

Graphical abstract

Systemic and pulmonary congestion by ultrasound and prognosis in heart failure: a pooled cohort analysis

Objective: Evaluate the prognostic value of the co-existence of pulmonary and systemic congestion

Methods:

8 zone Lung Ultrasound

In-patients n= 500

Terciles of B-lines:

Mild (T1 <16),

Moderate (T2 ≥16 to 29),

Severe (T3 ≥30)

Out-patients n = 335

Terciles of b-lines:

Mild (T1 <3),

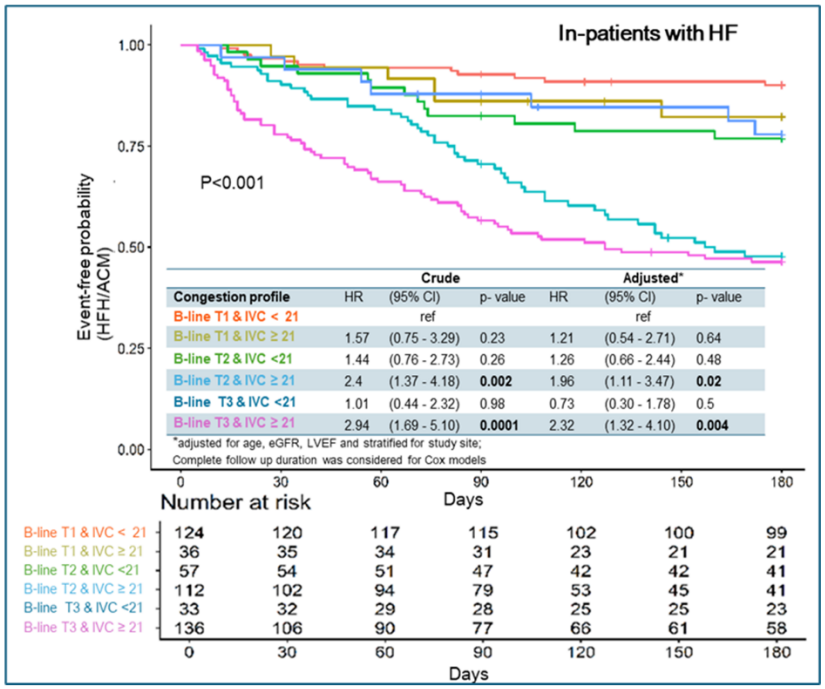
Moderate (T2 ≥3 to 12),

Severe (T3 ≥12).

Dilated IVC ≥21mm

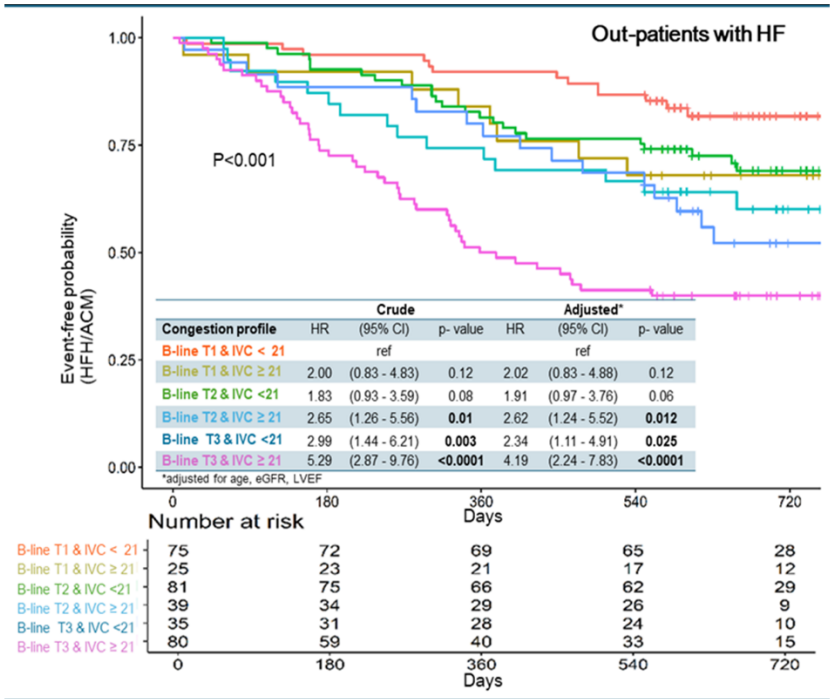
Conclusion: Amongst patients with HF, the combination of both pulmonary and venous congestion is associated with a worse prognosis than either sign of congestion alone.

Results: Association with HF hospitalizations and mortality



Congestion profile	Crude HR	(95% CI)	p- value	Adjusted* HR	(95% CI)	p- value
B-line T1 & IVC < 21	ref			ref		
B-line T1 & IVC ≥ 21	1.57	(0.75 - 3.29)	0.23	1.21	(0.54 - 2.71)	0.64
B-line T2 & IVC <21	1.44	(0.76 - 2.73)	0.26	1.26	(0.66 - 2.44)	0.48
B-line T2 & IVC ≥ 21	2.4	(1.37 - 4.18)	**0.002**	1.96	(1.11 - 3.47)	**0.02**
B-line T3 & IVC <21	1.01	(0.44 - 2.32)	0.98	0.73	(0.30 - 1.78)	0.5
B-line T3 & IVC ≥ 21	2.94	(1.69 - 5.10)	**0.0001**	2.32	(1.32 - 4.10)	**0.004**

*adjusted for age, eGFR, LVEF and stratified for study site;
Complete follow up duration was considered for Cox models



Congestion profile	Crude HR	(95% CI)	p- value	Adjusted* HR	(95% CI)	p- value
B-line T1 & IVC < 21	ref			ref		
B-line T1 & IVC ≥ 21	2.00	(0.83 - 4.83)	0.12	2.02	(0.83 - 4.88)	0.12
B-line T2 & IVC <21	1.83	(0.93 - 3.59)	0.08	1.91	(0.97 - 3.76)	0.06
B-line T2 & IVC ≥ 21	2.65	(1.26 - 5.56)	**0.01**	2.62	(1.24 - 5.52)	**0.012**
B-line T3 & IVC <21	2.99	(1.44 - 6.21)	**0.003**	2.34	(1.11 - 4.91)	**0.025**
B-line T3 & IVC ≥ 21	5.29	(2.87 - 9.76)	**<0.0001**	4.19	(2.24 - 7.83)	**<0.0001**

*adjusted for age, eGFR, LVEF

Data availability statement

The data that supports the findings of this study are available upon reasonable request from the corresponding author.

Conflict of interest: Dr Girerd is supported by the French National Research Agency Fighting Heart Failure (ANR-15-RHU-0004), the French PIA project Lorraine Université d'Excellence GEENAGE (ANR-15-IDEX-04-LUE) programs, and the Contrat de Plan Etat Région Lorraine and FEDER IT2MP; and has received honoraria From AstraZeneka, Bayer, Boehringer, Lilly, Novartis, and Vifor. Dr Gargani is supported by Italian Ministry of Health and Regione Toscana; and has received personal fees From GE Healthcare, Phillips Healthcare, and Caption Health. Dr. Pellicori has received personal fees from Caption Health.

All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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Characteristic	**N**	**T1 (0 to < 16) & IVC < 21,** N = 124[1]	**T1 (0 to < 16) & IVC ≥ 21,** N = 36[1]	**T2 (≥ 16 to < 30) & IVC < 21,** N = 57[1]	**T2 (≥ 16 to < 30) & IVC ≥ 21,** N = 112[1]	**T3 (≥ 30) & IVC < 21,** N = 33[1]	**T3 (≥ 30) & IVC ≥ 21,** N = 138[1]	**p-value**[2]
Age (years)	500	75 (67, 81)	73 (65, 80)	77 (65, 81)	79 (71, 83)	75 (69, 81)	81 (77, 86)	<0.001
Female	500	52 (42%)	12 (33%)	26 (46%)	49 (44%)	16 (48%)	64 (46%)	0.77
BMI (Kg/m^2)	460	26.8 (23.7, 30.4)	27.7 (24.1, 29.8)	28.3 (25.8, 30.1)	29.7 (26.4, 31.0)	26.0 (24.2, 28.7)	26.0 (24.0, 28.7)	<0.001
CAD	499	49 (40%)	14 (39%)	26 (46%)	50 (45%)	15 (45%)	69 (50%)	0.6
AF	499	36 (29%)	20 (56%)	12 (21%)	46 (41%)	4 (12%)	39 (28%)	<0.001
SBP (mmHg)	478	135 (120, 145)	121 (106, 140)	120 (110, 135)	130 (110, 139)	130 (110, 135)	130 (110, 140)	0.018
Heart rate (bpm)	475	71 (62, 86)	72 (66, 100)	83 (73, 89)	89 (84, 97)	94 (87, 99)	89 (86, 94)	<0.001
NYHA class III/IV	263	56 (47%)	22 (65%)	22 (65%)	29 (76%)	9 (64%)	24 (96%)	<0.001
Edema	498	32 (26%)	21 (58%)	25 (44%)	76 (68%)	15 (45%)	96 (70%)	<0.001
Rales	498	43 (35%)	15 (42%)	42 (74%)	92 (82%)	21 (64%)	126 (91%)	<0.001
Hemoglobin (g/dL)	393	12.80 (11.90, 13.80)	12.50 (11.75, 13.65)	11.90 (10.58, 12.73)	12.30 (10.85, 13.75)	12.90 (11.80, 13.45)	11.70 (10.60, 13.20)	<0.001
eGFR (mL/min/1.73 m2)	491	61 (49, 76)	56 (40, 67)	59 (45, 80)	49 (33, 67)	51 (38, 61)	48 (36, 62)	<0.001
NTproBNP (pg/mL)	193	1,576 (946, 2,647)	2,489 (1,516, 5,289)	3,712 (1,808, 5,231)	3,131 (1,978, 5,925)	3,302 (2,091, 4,852)	5,493 (3,179, 8,812)	<0.001
BNP (pg/mL)	366	473 (285, 691)	702 (368, 1,157)	753 (595, 926)	921 (590, 1,426)	746 (594, 1,017)	1,388 (870, 2,038)	<0.001
IVC (mm)	500	15.0 (13.0, 18.0)	25.0 (23.0, 27.0)	18.0 (15.0, 20.0)	24.0 (22.0, 25.0)	19.0 (17.0, 20.0)	24.0 (22.0, 26.0)	<0.001
LVEDD (mm)	268	54 (51, 60)	56 (54, 64)	55 (51, 61)	57 (51, 63)	55 (47, 63)	58 (51, 65)	0.66
LVEDV (ml)	243	141 (98, 178)	156 (121, 189)	145 (120, 173)	140 (109, 170)	150 (104, 185)	145 (113, 181)	0.81
LVEF (%)	499	39 (30, 50)	43 (25, 55)	40 (30, 50)	40 (30, 50)	43 (28, 55)	40 (25, 50)	0.89
HF type	499							0.79
HFmrEF		24 (19%)	8 (22%)	14 (25%)	32 (29%)	7 (21%)	29 (21%)	
HFpEF		29 (23%)	10 (28%)	12 (21%)	20 (18%)	10 (30%)	28 (20%)	
HFrEF		71 (57%)	18 (50%)	30 (54%)	60 (54%)	16 (48%)	81 (59%)	
PASP (mmHg)	417	42 (36, 49)	46 (40, 54)	42 (35, 48)	45 (40, 52)	44 (40, 54)	50 (44, 60)	<0.001
TAPSE (mm)	415	18.0 (16.0, 21.0)	14.0 (11.5, 18.0)	19.0 (16.0, 21.0)	18.0 (15.0, 21.0)	18.0 (16.0, 21.5)	17.0 (14.1, 20.0)	0.006
Beta Blocker	493	35 (29%)	15 (42%)	22 (40%)	57 (51%)	16 (48%)	81 (59%)	<0.001
ACEi / ARBs	493	85 (71%)	21 (58%)	42 (76%)	82 (73%)	31 (94%)	115 (83%)	0.003
MRA	277	54 (46%)	16 (44%)	22 (67%)	15 (34%)	8 (53%)	17 (55%)	0.1
CRT	254	30 (26%)	11 (34%)	11 (33%)	9 (24%)	4 (29%)	11 (48%)	0.38

Diuretics	493	89 (75%)	33 (92%)	51 (93%)	104 (93%)	27 (82%)	127 (92%)	<0.001

[1] Median (IQR); n (%); Mean (SD); Range

[2] Kruskal-Wallis rank sum test; Pearson's Chi-squared test; Fisher's exact test

BMI: Body Mass Index; CAD: Coronary Artery Disease; AF: Atrial Fibrillation; SBP: Systolic Blood Pressure; HR: Heart Rate; NYHA: New York Heart Association; eGFR: estimated Glomerular Filtration Rate; IVC: inferior vena cava diameter; LVEDD: Left Ventricular end Diastolic Diameter; LVEDV: Left Ventricular end Diastolic Volume; LVEF: Left Ventricular Ejection Fraction; HFmrEF: HF with mildly-reduced Ejection Fraction; HFpEF: HF with preserved Ejection Fraction; HF with reduced Ejection Fraction; LAVi: Left Atrial Volume index; TAPSE: Tricuspid Annular Plane Systolic Excursion; ACEi: Angiotensin Converting Enzyme inhibitor; ARA: Angiotensin Receptor Antagonist; MRA: Mineralocorticoid Receptor Antagonist; CRT: Cardiac Resynchronization, Therapy

Table1 : Baseline characteristics according to congestion status by ultrasound in in-patients cohorts.

Characteristic	**N**	**T1 & IVC <21**, N = 75[1]	**T1 & IVC ≥21**, N = 25[1]	**T2 & IVC <21**, N = 81[1]	**T2 & IVC ≥ 21**, N = 39[1]	**T3 & IVC <21**, N = 35[1]	**T3 & IVC ≥ 21**, N = 80[1]	**p-value**[2]
Age (years)	335	72 (67, 80)	69 (60, 78)	71 (62, 78)	76 (67, 83)	77 (74, 83)	81 (77, 85)	<0.001
Female	335	28 (37%)	7 (28%)	34 (42%)	9 (23%)	11 (31%)	23 (29%)	0.28
BMI (Kg/m^2)	335	31 (27, 33)	31 (26, 39)	29 (25, 34)	31 (28, 34)	27 (24, 30)	28 (24, 30)	<0.001
Smoker	335	11 (15%)	4 (16%)	14 (17%)	3 (7.7%)	2 (5.7%)	9 (11%)	0.49
Diabetes	335	21 (28%)	13 (52%)	20 (25%)	10 (26%)	12 (34%)	20 (25%)	0.13
HTN	335	33 (44%)	12 (48%)	42 (52%)	26 (67%)	22 (63%)	49 (61%)	0.12
CAD	335	32 (43%)	16 (64%)	38 (47%)	15 (38%)	20 (57%)	42 (53%)	0.25
COPD	335	13 (17%)	3 (12%)	19 (23%)	4 (10%)	9 (26%)	17 (21%)	0.42
AF	335	22 (29%)	14 (56%)	22 (27%)	29 (74%)	11 (31%)	64 (80%)	<0.001
SBP (mmHg)	335	134 (122, 152)	133 (125, 138)	134 (119, 149)	138 (125, 154)	145 (124, 159)	135 (121, 161)	0.39
HR (bpm)	335	67 (60, 78)	67 (64, 72)	70 (63, 77)	70 (60, 80)	74 (67, 83)	74 (65, 80)	0.061
NYHA III/IV	335	10 (13%)	5 (20%)	10 (12%)	12 (31%)	8 (23%)	39 (49%)	<0.001
Edema	335	13 (17%)	7 (28%)	19 (23%)	15 (38%)	11 (31%)	51 (64%)	<0.001
Rales	335	0 (0%)	2 (8.0%)	3 (3.7%)	2 (5.1%)	5 (14%)	28 (35%)	<0.001
Hemoglobin (g/dL)	335	13.2 (12.3, 14.4)	13.3 (12.4, 15.2)	13.5 (11.8, 14.4)	13.3 (12.3, 14.2)	13.0 (12.3, 14.1)	12.5 (11.3, 13.6)	0.013
eGFR (mL/min/1.73 m2)	334	65 (46, 80)	68 (55, 78)	63 (46, 85)	63 (39, 76)	63 (47, 77)	57 (40, 65)	0.13

NTproBNP (pg/mL)	333	620 (293, 1,110)	1,563 (475, 2,363)	665 (223, 1,579)	1,413 (942, 2,078)	1,531 (865, 2,734)	3,213 (1,886, 5,337)	<0.001
IVC (mm)	335	16.0 (15.0, 18.0)	22.0 (21.0, 24.0)	17.0 (15.0, 19.0)	23.0 (22.0, 25.5)	18.0 (17.0, 19.1)	26.0 (23.0, 29.0)	<0.001
LVEDD (cm)	335	5.20 (4.80, 5.90)	6.00 (5.40, 6.40)	5.20 (4.60, 6.10)	5.10 (4.80, 5.75)	5.60 (4.80, 6.25)	5.70 (5.10, 6.10)	0.034
LVEDV (ml)	335	136 (98, 184)	184 (113, 215)	132 (109, 198)	126 (100, 157)	155 (114, 219)	149 (115, 194)	0.082
LVEF %	335	48 (36, 57)	39 (34, 55)	46 (35, 55)	52 (42, 58)	44 (33, 53)	45 (35, 55)	0.25
HF type	335							0.14
HFmrEF		18 (24%)	1 (4.0%)	22 (27%)	6 (15%)	7 (20%)	19 (24%)	
HFpEF		31 (41%)	11 (44%)	29 (36%)	24 (62%)	13 (37%)	30 (38%)	
HFrEF		26 (35%)	13 (52%)	30 (37%)	9 (23%)	15 (43%)	31 (39%)	
LAD (cm)	335	4.00 (3.75, 4.60)	4.60 (4.30, 4.80)	4.00 (3.50, 4.60)	4.60 (3.90, 5.00)	4.30 (3.70, 4.75)	4.80 (4.48, 5.23)	<0.001
LAV (ml)	335	72 (52, 88)	104 (82, 111)	77 (52, 91)	88 (74, 117)	76 (68, 102)	109 (92, 148)	<0.001
LAVi (ml/m^2)	335	35 (29, 45)	45 (39, 55)	39 (26, 47)	43 (37, 59)	43 (36, 55)	59 (49, 74)	<0.001
TAPSE (mm)	327	22.0 (16.3, 25.8)	18.0 (15.0, 21.5)	21.0 (16.0, 24.0)	19.0 (14.5, 21.5)	19.0 (15.5, 22.0)	16.0 (13.0, 20.0)	<0.001
Mitral regurgitation grade (%)	335							<0.001
None		54 (72.0)	7 (28.0)	48 (59.3)	20 (51.3)	14 (40.0)	16 (20.0)	
Mild		21 (28.0)	16 (64.0)	32 (39.5)	18 (46.2)	17 (48.6)	55 (68.8)	
Mod to severe		0 (0.0)	2 (8.0)	1 (1.2)	1 (2.6)	4 (11.4)	9 (11.2)	
Tricuspid regurgitation grade (%)	335							<0.001
None		56 (74.7)	9 (36.0)	59 (72.8)	18 (46.2)	20 (57.1)	15 (18.8)	
Mild		19 (25.3)	15 (60.0)	22 (27.2)	20 (51.3)	15 (42.9)	54 (67.5)	
Mod to severe		0 (0.0)	1 (4.0)	0 (0.0)	1 (2.6)	0 (0.0)	11 (13.8)	
Beta Blocker	335	66 (88%)	20 (80%)	68 (84%)	34 (87%)	28 (80%)	65 (81%)	0.78
ACEi /ARB	335	70 (93%)	22 (88%)	74 (91%)	33 (85%)	28 (80%)	57 (71%)	0.002
MRA	335	36 (48%)	14 (56%)	45 (56%)	16 (41%)	16 (46%)	36 (45%)	0.62
CRT	335	6 (8.0%)	2 (8.0%)	3 (3.7%)	3 (7.7%)	5 (14%)	9 (11%)	0.39
Diuretics	335	51 (68%)	18 (72%)	55 (68%)	29 (74%)	26 (74%)	72 (90%)	0.017
Loop Diuretics~	335							0.052
0		24 (32%)	7 (28%)	26 (32%)	10 (26%)	9 (26%)	8 (10%)	

≤40 mg/day	33 (44%)	11 (44%)	37 (46%)	16 (41%)	16 (46%)	31 (39%)
>40 up to 80 mg/day	10 (13%)	4 (16%)	11 (14%)	7 (18%)	6 (17%)	22 (28%)
>80 mg/day	8 (11%)	3 (12%)	7 (8.6%)	6 (15%)	4 (11%)	19 (24%)

[1] Median (IQR); N (%); Mean (SD); Range

[2] Kruskal-Wallis Rank Sum Test; Pearson's Chi-Squared Test; Fisher's Exact Test

BMI: Body Mass Index; HTN: Hypertension; CAD: Coronary Artery Disease; COPD: Chronic Obstructive Pulmonary Disease; AF: Atrial Fibrillation; SBP: Systolic Blood Pressure; HR: Heart Rate; NYHA: New York Heart Association; eGFR: estimated Glomerular Filtration Rate; IVC: inferior vena cava diameter;LVEDD: Left Ventricular end Diastolic Diameter; LVEDV: Left Ventricular end Diastolic Volume; LVEF: Left Ventricular Ejection Fraction; HFmrEF: HF with mildly-reduced Ejection Fraction; HFpEF: HF with preserved Ejection Fraction; HF with reduced Ejection Fraction; LAD: Left Atrium Diameter; LAV: Left Atrium Volume; LAVi: Left Atrial Volume index; TAPSE: Tricuspid Annular Plane Systolic Excursion; ACEi: Angiotensin Converting Enzyme inhibitor; ARA: Angiotensin Receptor Antagonist; MRA: Mineralocorticoid Receptor Antagonist; ~ dose reported as furosemide/equivalent

Table 2: Baseline characteristics according to congestion status by ultrasound in the out-patient cohort.

Supplementary data

Supplemental Table1: Crude and adjusted risk estimates for IVC diameter and B-lines as continuous variable.

Cohort	Variable	Crude HR (95%CI)	P-value	Adjusted HR (95%CI)**	P-value
Admission*	B-line continuous	1.01 (1.00 - 1.03)	**0.023**	1.01 (1.00 - 1.02)	0.14
	IVC continuous	1.10 (1.07 - 1.13)	**<0.0001**	1.10 (1.07 - 1.14)	**<0.0001**
	B-line +	1.01 (0.99 - 1.02)	0.26	1.00 (0.99 - 1.02)	0.74
	IVC	1.10 (1.07 - 1.13)	**<0.0001**	1.10 (1.07 - 1.14)	**<0.0001**
OPD	B-line continuous	1.02 (1.01 - 1.02)	**<0.0001**	1.01 (1.01 - 1.02)	**0.0007**
	IVC continuous	1.08 (1.06 - 1.10)	**<0.0001**	1.09 (1.06 - 1.11)	**<0.0001**
	B-line +	1.01 (1.01 - 1.02)	**0.001**	1.01 (1.00 - 1.02)	0.053
	IVC	1.07 (1.05 - 1.10)	**<0.0001**	1.08 (1.05 - 1.11)	**<0.0001**

4.2.2.1. Learning forward

In this study we evaluated the relatively advanced imaging modalities of pulmonary and systemic congestion assessment. We demonstrated that not only patients have different degrees of pulmonary and systemic congestion, these interactions influenced the prognosis of HF patients. **This paper was submitted to the International Journal of cardiology and is under review**.

In all the previous studies presented in this thesis, we established that a more comprehensive and accurate congestion assessment can improve risk stratification. However, in all these studies, presence of congestion was defined using pre-determined cut-offs. These cut-offs were either chosen based on published literature or through statistical methods like tertiles. While the cut-offs are required for analysis, they may not resonate clinically. For example, a patient with number of B-line 14 vs 16 may not be clinically very different but one would be classified as mild congestion whereas other as moderate congestion.

In all of the previous studies, we were restricted to few congestion parameters which limits our ability to have a complete picture of congestion. For instance, our first paper focused on the prognostic utility of B-lines. The second paper combined clinical congestion, pulmonary congestion and natriuretic peptides while the third paper assessed the prognostic utility of combined assessment of venous and pulmonary congestion measured using on ultrasound. All these paper were assessing one or two parameters of congestion concomitantly. **We could classify congestion into sub-groups based on severity of congestion but classifying patients in congestion profiles while considering all the dimensions of congestion is not feasible using conventional statistical approach**. Moreover, in the previous studies, we did not explore the proteins pathways associated with congestion profiles. **These studies highlighted the need for a more comprehensive approach**. Each marker provides a different piece of puzzle; B-lines provide information on pulmonary congestion, IVC diameter on venous and organ congestion, natriuretic peptides on myocardial stretch and clinical congestion scores capture systemic signs. Hence, in the next paper we chose to analyze several dimensions of congestion using machine-learning approaches to identify congestion phenotypes. Additionally, we explored the biological underpinnings of the congestion profiles using complex networks.

4.3. Identification of congestion profiles using clustering analysis

4.3.1. Identifying congestion phenotypes using unsupervised machine learning in acute heart failure

The 3 previous articles presented in this thesis show that clinically derived congestion classification provides improved risk stratification in HF. However, only a few clinical variables can be considered in the traditional approaches. On the other hand, machine learning based approaches can analyze a large array of data-points on demographics, comorbidities, imaging and biological data. Thus, enabling the identification of homogenous congestion profiles of HF within a heterogenous population. In this analysis we sought to elucidate the underlying pathophysiology of congestion phenotypes based on pulmonary and systemic congestion at both the tissue (PTC: pulmonary tissue congestion, STC: systemic tissue congestion) and intravascular (PIVC: pulmonary intravascular congestion, SIVC: systemic intravascular congestion) level in patients with acute HF.

We analyzed 19 clinical, laboratory and echocardiographic congestion parameters using LCM model of clustering analysis. We identified 5 different congestion profiles in Nancy-HF cohort (n = 741). These profiles were labeled as follows: 1) PTC-dilated left ventricle (LV), 2) PTC-HFpEF, 3) PTC, STC-atrial fibrillation (AF), 4) PIVC-dilated left atrium (LA) and LV and 5) Global congestion. Subsequently, we developed an RF model to predict cluster class and validated the RF model in BIOSTAT-CHF cohort (n = 4254). We also analyzed 363 proteins to study the association between each protein and congestion phenotype. Thereafter, network analysis was conducted to identify underlying pathways. We found that ‘PTC-HFpEF’ was related to lipid, protein and angiotensin metabolism, ‘PTC, STC-AF’ was related to kinase-mediated signaling, extracellular matrix organization and TNF-regulated cell death, while ‘PIVC-dilated LA & LV’ was related to kinase-mediated signaling and hemostasis.

We also found that the risk of composite outcome was higher in ‘PTC, STC-AF’ and ‘Global’ congestion phenotypes [adjusted HR:1.74(1.13-2.67) and 2.41(1.60-3.63), respectively] compared to PTC-dilated LV’ phenotype in Nancy-HF. Similar pattern of risk association was observed in clusters identified in BIOSTAT-CHF cohort.

Our findings provide insights into the biological pathways and how each congestion profile is different. The protein profiles associated with each phenotype could be the potential therapeutic targets for managing congestion. In addition, we developed the RF model (Ai-Cong-HF: https://cic-p-nancy.fr/ai-cong-hf/) which may be used to identify the congestion phenotype for clinical and research purpose.

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ORIGINAL ARTICLE

Identifying congestion phenotypes using unsupervised machine learning in acute heart failure

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Abstract

Background and aims: Data-driven clustering techniques may improve heart failure (HF) categorization and provide prognostic insights. The present study aimed to elucidate the underlying pathophysiology of acute HF phenotypes based on pulmonary and systemic congestion at both the tissue (PTC:pulmonary tissue congestion, STC:systemic tissue congestion) and intravascular (PIVC:pulmonary intravascular congestion, SIVC:systemic intravascular congestion) level and to assess the association of identified phenotypes with a composite outcome of HF hospitalization and death.

Methods: Nineteen clinical, laboratory, and echocardiographic congestion markers were analyzed using clustering techniques to identify phenotypes in patients with worsening HF in the Nancy-HF cohort (n=741), followed by validation of the clustering model in the BIOSTAT-CHF cohort (n=4254). Network analysis was conducted using 363 proteins to identify underlying biological pathways.

Results: Five congestion phenotypes were identified: 1)PTC-dilated left ventricle (LV), 2)PTC-HFpEF, 3)PTC, STC-atrial fibrillation (AF), 4)PIVC-dilated left atrium (LA) and LV and 5)Global congestion. Compared to the 'PTC-dilated LV' phenotype, the risk of composite outcome was higher

in ‘PTC, STC-AF’ and ‘Global’ congestion phenotypes [adjusted HR:1.74(1.13-2.67) and 2.41(1.60-3.63), respectively]. In BIOSTAT-CHF, ‘Global’ congestion phenotype was associated with significantly higher risk [HR:1.64(1.04-2.58)]. In network analysis, the immune response pathway was linked to all phenotypes. ‘PTC-HFpEF’ was related to lipid, protein and angiotensin metabolism, ‘PTC, STC-AF’ was related to kinase-mediated signaling, extracellular matrix organization and TNF-regulated cell death, while ‘PIVC-dilated LA & LV’ was related to kinase-mediated signaling and hemostasis.

Conclusion: In worsening HF, clustering techniques identified clinical congestion profiles associated with both long-term clinical risk and differences in biomarkers, suggesting potential different underlying pathophysiologies. These clusters can be applied using the available online model to identify phenotypes as well as associated risks.(https://cic-p-nancy.fr/ai-cong-hf/).

Keywords: heart failure phenotypes; congestion; protein biomarkers; random forest; unsupervised clustering.

List of abbreviations:

ACEi	Angiotensin Converting Enzyme inhibitor
AF	Atrial Fibrillation
ARA	Angiotensin Receptor Antagonist
ARNi	Angiotensin and Neprilysin Inhibitor
BMI	Body Mass Index
BNP	Brain Natriuretic Peptide
CAD	Coronary Artery Disease
COPD	Chronic Obstructive Pulmonary Disease
eGFR	estimated Glomerular Filtration Rate
FDR	False Discovery Rate
Hb	Hemoglobin
HF	heart failure
HFmrEF	HF with mildly-reduced Ejection Fraction
HFpEF	HF with preserved Ejection Fraction
HFrEF	HF with reduced Ejection Fraction
HR	Heart Rate
HTN	Hypertension
JVD	Jugular Venous Distention
LA	Left Atrium
LAVi	Left Atrial Volume index
LOD	Limit Of Detection
LV	Left Ventricle
LVEDVi	Left Ventricular End Diastolic Volume index
LVEF	Left Ventricular Ejection Fraction
LVMi	Left Ventricular Mass index

MRA	Mineralocorticoid Receptor Antagonist
NYHA	New York Heart Association
PASP	Pulmonary artery systolic pressure
SBP	Systolic Blood Pressure
TAPSE	Tricuspid Annular Plane Systolic Excursion
AHF	Acute Heart Failure
PIVC	Pulmonary intravascular Congestion
PTC	Pulmonary Tissue Congestion
Pulm	Pulmonary
RF	Random Forest
SGLT2i	Sodium Glucose Cotransporter 2 inhibitor
SIVC	Systemic Intravascular Congestion
STC	Systemic Tissue Congestion
XGBoost	eXtreme Gradient Boosting

Introduction

Congestion is a key feature of acute heart failure (AHF) that influences both short- and long-term prognosis (1). Congestion may be causally linked with deteriorating heart function by activating inflammatory, neuro-hormonal and oxidative stress pathways (2-4). De-congestion is one of the key therapeutic goals in acute and chronic HF (5). However, available decongestion therapies remain inadequate (6). An optimal combination of ARNi, beta-blockers, mineralocorticoid antagonist and SGLT2i improves prognosis in HF patients (7, 8). Yet, this combination therapy remains inadequate in a sizeable population, and its implementation is often limited by presence of clinical inertia or comorbidities such as hypotension, electrolyte imbalance, renal dysfunction (9, 10). Approximately 30-45% of AHF patients are re-admitted or die within 1 year of discharge (11, 12). Moreover, a large heterogeneity in congestion types, severity and distribution is suggested to be one of the factors behind inadequate treatment response (2). Identifying homogenous "phenotypes" amongst the heterogeneous HF population hence appears as a logical first step towards individualization of therapy from the current "one size fits all" approach. Furthermore, the ESC-HF guidelines 2021 suggest that a better phenotyping of AHF may improve treatment and prognosis (11). Further compounding the issue, the pathophysiology of congestion scenarios has yet to be elucidated, likely due to the absence of easily identifiable phenotypes.

Unsupervised machine learning approaches recently identified distinct "phenotypes" of AHF that go beyond the "wet/dry" and/or LVEF-based classification. These distinct AHF phenotypes differ both in their prognosis and response to available therapies. (13-15) Clustering approaches can support clinical decision-making regarding treatment individualization through the identification of AHF phenotypes by analyzing a multitude of data points on demographics, comorbidities, echocardiographic findings and/or circulating protein biomarkers. However, studies assessing congestion phenotypes of AHF based on clinical and echocardiographic congestion variables are currently sparse (14, 16). Given the impact of congestion on patient survival, we hypothesized that

congestion markers-based phenotyping may be more propitious in classifying AHF patients and discovering treatment targets.

In the present study, we sought to unveil the complex pathophysiology of congestion phenotypes in patients with AHF by applying a clustering analysis to categorize these phenotypes based on a comprehensive array of variables, including clinical signs, symptoms, blood tests, and echocardiographic data. To validate and broaden our understanding of these phenotypes, the resulting model was applied in a validation cohort and a network analysis was conducted on circulating protein data to explore the biological underpinnings, assessing their prognostic value by examining associations with clinical outcomes.

Methods

Derivation cohort

For cluster identification, patients with HF admitted for worsening HF included in the Nancy-HF cohort (n = 741) from January 2015 to May 2019 at the Nancy University Hospital were analyzed. Patients were identified using ICD-9 codes related to HF. The details of the study design and population have been published previously (17).

Variable selection and identification of clusters using machine learning

Variables typically used for assessing and quantifying congestion in routine clinical practice were considered. In addition, variables potentially impacting congestion assessment were also included for model building. Variables with greater than 30% missing values were discarded from the analysis, leading to removal of the A wave, e' mean and E/e' mean variables. However, E/A was retained for analysis despite 41% missing values given its value as an important indicator of diastolic left ventricular dysfunction. And, E/e'lateral was used instead of E/e' mean.

The variables used in the final clustering model included congestion variables, namely rales, peripheral edema, jugular venous distension (JVD), hemoglobin, BNP/NT-proBNP z-score, left atrial volume index (LAVi), pulmonary artery systolic pressure (PASP), E/e' lateral and E/A ratio, as well as variables necessary for adequately assessing the prognostic impact of congestion, or the contextual interpretation of congestion, namely hypertension, coronary artery disease (CAD), known history of atrial fibrillations (AF), heart rate, systolic blood pressure (SBP), estimated glomerular filtration rate (eGFR), left ventricular ejection fraction (LVEF), left ventricular mass index (LVMi), left ventricular end-diastolic volume index (LVEDVi), and tricuspid annular plane systolic excursion (TAPSE). Of note, AF is a good example of a variable having a sizeable impact on how echocardiographic variables are interpreted – having AF and other congestion-related variables as input variables in the clustering approach allows more adequate deciphering of the homogeneous groups by considering covariates that modify the interpretation of congestion variables. BNP z score was used to harmonize data with NTproBNP data available in the validation cohort.

The clustering analysis was conducted using the VarSelLCM package in R and the optimal number of clusters was identified using the Bayesian Information Criterion (BIC). Random Forest (generating 250 trees), XGBoost, Support Vector Machine and Decision Tree prediction models (7 instances per leaf) with 10-fold cross-validation were subsequently trained in Weka and R software and compared for their performance. Prediction models included age, sex, and BMI, as well as all variables used to identify the aforementioned clusters.

After identifying the clusters, a labelling process was applied to characterize clusters based on congestion phenotype. The labels align with the framework proposed by recent reviews focusing on congestion, which classify patients according to the regional (pulmonary vs. systemic) and compartmental (intravascular vs. tissue) distribution of congestion (18, 19). The following features were considered:

Pulmonary Tissue Congestion (PTC): Characterized by a high frequency of rales.

Systemic Tissue Congestion (STC): Defined by a high frequency of peripheral edema.

Systemic Intravascular Congestion (SIVC): Identified by a high frequency of JVD.

Pulmonary Intravascular Congestion (PIVC): Associated with elevated BNP levels, high pulmonary artery systolic pressure, increased left atrial volume index (LAVi), and echocardiographic markers such as E/e' lateral and E/A.

Clusters were labelled as having one or several of these congestion features.

Validation of the random forest model in BIOSTAT-CHF

The random forest model derived in the Nancy-HF cohort was validated in the BIOSTAT-CHF cohort (n= 4254, pooled data of BIOSTAT-CHF index and BIOSTAT-CHF validation cohorts), a prospective observational cohort that included patients with signs and symptoms of new-onset or worsening HF (20). In BIOSTAT-CHF, the LAV was calculated from LA diameter using the linear equation derived in the STANISLAS cohort (21). E/e'lateral, TAPSE and PASP were unavailable and therefore considered missing in the analysis.

Protein biomarkers in BIOSTAT-CHF

To better identify the pathophysiological background of congestion clusters, 368 protein biomarkers from the OLINK® CVD II, CVD III, Immune Response and Oncology II panels were assessed in BIOSTAT-CHF. Of the 368 proteins assessed in the validation cohort, 5 protein biomarkers (TNF r1, Cstb, Pi3, Klk6, Azul1) were removed as >10% of protein values were below the limit of detection (LOD). A multinomial regression analysis was next conducted to identify proteins significantly associated with each phenotype, with overall p-values corrected for false discovery rate (FDR) as described in the Statistical analysis section.

Complex network analysis

The proteins that were uniquely associated with any of the congestion phenotypes were selected for network analysis. Protein annotations were extracted from the FHF graph knowledge

box (FHF-GKBox) (22) from which protein–pathway relationships were extracted from Reactome (version 69) (23). The FHF-GKBox was searched using query patterns, which are templates predefined in the Cypher Query language, allowing to systematically extract sub-graphs from a Neo4J graph database. Two query patterns were employed, depending on the number of proteins significantly associated with each phenotype: firstly, a biomarker-pathway-biomarker query pattern was defined and used to retrieve pathways linking at least two distinct biomarkers; secondly, a pathway-biomarker pattern was employed to identify all pathways that were significantly overrepresented. The resulting sub-graph encompassing all identified pathways and related biomarkers was automatically visualized using the Cytoscape software (24).

Outcome in the Nancy-HF and BIOSTAT-CHF cohorts

The outcome was a composite of hospitalization for HF or death in the derivation cohort and the validation cohort.

Statistical analysis

For the descriptive analyses, continuous variables are expressed as mean ± standard deviation (SD) for normally distributed data or as median (Q1-Q3) for skewed data. Categorical variables are expressed as proportions (%). Comparison of baseline characteristics was performed using ANOVA or the Kruskal–Wallis test as required.

Distribution pattern of outcome was examined across all identified phenogroups, stratified by age, sex and LVEF category. Survival probabilities were plotted using Kaplan-Meier curves. The phenotype with the best survival was considered as reference. Cox proportional hazard models for identified phenotypes were used to obtain unadjusted and adjusted hazard ratios. The variables for adjusted model 1 were age, sex, BMI, SBP, LVEF, eGFR while variables for model 2 were: model1 + BNP/NT-proBNP z scores. In the Nancy-HF cohort, Cox proportion hazard models were further adjusted for medications (ACEi/ARBs, beta-blockers and aldosterone) to assess the impact of

treatment on prognosis for the identified phenotypes. The association of protein biomarkers with identified phenotypes was assessed using a multinomial logistic regression model. The p-values for multiple comparisons were corrected for FDR (at 5%) using the Benjamini-Hochberg correction.

A two-tailed p-value <0.05 was considered significant. The analyses were conducted with R (R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) and Weka software packages © (version 3.8.6)

Results

In Nancy-HF, the mean age of study participants was 75 (±12) years and included 50% women. More than 80% of the patients had hypertension and mean LVEF was 49.0 (±15.6)%.

Congestion phenotypes identified in the Nancy-HF cohort

Five different congestion phenotypes of patients with AHF were identified in the Nancy-HF cohort. The top discriminative variables in the clustering analysis were BNP/NT-proBNP z-score, LVEF, LVEDVi, LAVi and E/A ratio **(Supplementary Figure 1S)**. Baseline characteristics of the identified phenotypes are presented in **Table 1 and Figure 1.**

The key characteristics of the 5 phenotypes are as follows:

- 'Pulm-tissue congestion with dilated LV' (PTC-dilated LV; n = 175): patients with a high likelihood of CAD, frequent rales, all ranges of LVEF (range 16-68%), high LVMi and high LVEDVi.
- 'Pulm tissue congestion with HFpEF' (PTC-HFpEF; n = 137): patients with high SBP, frequent rales, predominantly HFpEF (LVEF range 45-77%) with small to normal LVEDVi.

- ‘Pulm tissue and Syst tissue congestion with AF’ (PTC, STC-AF; n = 167): high likelihood of AF, frequent rales and peripheral edema, all ranges of LVEF (range 21-82%), high E/A, lower TAPSE and small LVEDVi.
- ‘Pulm IV congestion with dilated LA & LV’ (PIVC-dilated LA & LV; n = 138): mostly men, predominantly HFrEF (LVEF range 11-70), highest BNP, low TAPSE, dilated LA and LV.
- ‘Global’ congestion (n = 124): frequently raised JVD, peripheral edema, predominantly HFpEF and HFmrEF (LVEF range 38 to 81%), severely dilated LA, lower TAPSE and high PASP.

Models predicting cluster membership

The random forest (RF) model exhibited 77.3% sensitivity, 93.7% specificity, and 79.4% precision **(Supplementary Figure 2S)**. The decision tree and support vector machine models had a lower predictive capacity than the RF model (~68% sensitivity, 92% specificity, and 68% precision **(Supplementary Figures 3S, 4S and 5S)**. XGBoost had similar prediction parameters as RF model however, cluster characteristics of phenotypes in the validation cohort were different from derivation cohort (data not shown). Cluster memberships were hence predicted using RF in the subsequent steps of the analysis in the BIOSTAT-CHF (validation) cohort.

Predicted congestion phenotypes in BIOSTAT- CHF

In BIOSTAT-CHF, nearly 30% of participants were women and mean age was 70 (±12) years. The RF model (constructed in Nancy-HF) identified similar congestion profiles in BIOSTAT-CHF **(Supplementary Table 1S).**

In the validation cohort, the majority of patients were classified as ‘PTC-dilated LV’ (n = 2796/4254, 65.7%), followed by ‘PIVC-dilated LA & LV’ (n = 956/4254, 22.5%) and ‘PTC, STC-AF’ (n = 384/4254). Fewer patients were identified in the ‘PTC-HFpEF’ (n = 83) and ‘Global’

congestion phenotypes (n=35). The distribution pattern of clinical characteristics, congestion markers and echocardiography variables in the validation cohort was similar to the derivation cohort.

Pathways linked to protein biomarkers associated with congestion phenotypes

Compared to 'PTC-dilated LV', 287 proteins were significantly associated with other clusters **(Supplementary Figure 6S and Supplementary Table 2S)**. Of the 287 proteins, 25 proteins were associated with all of the clusters, while some biomarkers were identified in only one cluster: 3 proteins with 'PTC-HFpEF' (all downregulated), 7 proteins significantly associated with 'PTC, STC-AF' (2 upregulated: 5 downregulated), 49 proteins significantly associated with 'PIVC-dilated LA & LV' (32 upregulated: 17 downregulated) and 1 significantly associated with the 'Global' congestion (upregulated) phenotype **(Supplementary Table 2S)**.

All protein-biological pathways characterized via the network analysis are presented in **Figure 2**. 'PTC-HFpEF' was associated with pathways regulating the immune system and metabolism of angiotensin, proteins and lipids. DDX58 emerged as a central node regulating these latter pathways in this phenotype. 'PTC, STC-AF' was associated with the innate immune system, extracellular matrix organization and kinase-mediated signaling. The 'PIVC-dilated LA & LV' phenotype was associated with kinase-mediated signaling, TNF-regulated cell death, immune system, hemostasis and vesicle-mediated transport. ERBB2, FASLG, TRAF2 emerged as the key nodes in this phenotype. The 'Global' congestion phenotype was associated with only Gal-4 protein and no associated protein pathways were identified in the Reactome database.

Association of congestion phenotypes with survival in the Nancy-HF and BIOSTAT-CHF cohorts

In Nancy-HF, 361 events (155 HHF and 272 deaths) were observed over a median follow-up of 17.1 [6.3, 29.0] months. Stratified analyses revealed that, as expected, the distribution of phenogroups varied across age, sex, and LVEF category strata. Importantly, the risk stratification properties of the phenogroups were largely preserved within these strata (Supplementary Figure 7S).

‘PTC-dilated LV’ and ‘PTC-HFpEF’ had a higher survival probability compared to the other phenotypes **(Figure 3a),** followed by ‘PTC, STC-AF’ and ‘PIVC-dilated LA & LV’ whereas the ‘Global’ congestion phenotype had the worst prognosis. The adjusted association (M1) with primary outcome was HR = 1.50 (1.01 - 2.23), p = 0.044 for ‘PTC, STC-AF’, HR 1.37 (0.94 - 2.00), p = 0.099 for ‘PIVC-dilated LA & LV’ (**Table 2**) and HR = 2.38 (1.61 - 3.52), p <0.0001 for ‘Global’ congestion compared to ‘PTC-dilated LV’. Similar results were observed after adjusting for BNP z-score **(Table2)** and medications **(Supplementary Table 3s).**

In the BIOSTAT-CHF cohort, the ‘PTC-dilated LV’ and ‘PTC-HFpEF’ phenotypes had a better survival prognosis, followed by the ‘PTC, STC-AF’, ‘PIVC-dilated LA & LV’ and ‘Global’ congestion phenotypes **(Figure 3b)** over a median follow-up time of 18.6 [8.5, 27.3] months. The risk of primary outcome was significantly higher in ‘PIVC-dilated LA & LV’ and ‘Global’ congestion in Model M1 [HR =1.65 (1.46 - 1.87)), p < 0.0001 and HR = 1.82 (1.17 - 2.83), p = 0.008), respectively] but not in the ‘PTC, STC-AF’ phenotype. **(Table 2).**

Discussion

The unsupervised machine learning analysis applied in the present study identified 5 distinct phenotypes based on clinical characteristics and type and severity of congestion markers in AHF patients. These phenotypes could be predicted effectively using an RF model (**Graphical abstract**). More importantly, each congestion profile had a relatively distinct protein profile and involved different biological pathways, hence shedding new light on our understanding of congestion in acute settings. Our findings suggest that classifying AHF using a machine learning model based on clinical and congestion parameters could prove useful in categorizing the patients and predicting risk in future studies aimed at exploring individualized therapies in light of the different biological backgrounds related to each congestion phenotype.

Congestion phenotypes in AHF

Routinely available clinical variables (hypertension, CAD, AF, rales, peripheral edema, JVD, heart rate, SBP), laboratory variables (hemoglobin, eGFR, BNP) as well as echocardiography variables (LVEF, LVMi, LAVi, LVEDVi, TAPSE, PASP, E/e' lateral and E/A ratio) were selected to conduct the clustering analysis herein. Demographic characteristics such as age, sex or BMI were not included in order to maintain focus on identifying the congestion profiles as these variables significantly influence cluster designation. Factors such as CAD, AF, LVMi and TAPSE were considered as they provide insights into the etiopathogenesis of HF, prognosis and aid in adequate assessment of congestion. Nevertheless, the phenotypes obtained after clustering analysis were, to some extent, congruous with certain phenotypes reported in previous studies, including demographic profiles and other comorbidities (25, 26). The phenogroups 'PTC-dilated LV', PTC, STC -AF' and 'Global' congestion were comparable to profiles identified as 'Young', 'Elderly/AF' and 'Diabetes and CKD' (despite the differences in age and frequency of comorbidity) in a recent study which identified AHF phenotypes based on comorbidities (25). Moreover, these phenogroups had the best, intermediate and worst prognosis respectively in both studies. Our findings suggest that different AHF phenotypes may overlap with congestion patterns, possibly due to the impact of comorbidities on the development of congestion (e.g., sodium avidity).

The comorbidity and echocardiographic profiles of the 'PTC-dilated LV', 'PTC, STC-AF' and 'PIVC-dilated LA & LV' phenotypes are partially concordant with the clusters identified in previous studies assessing a broad spectrum of HF patients (16, 27, 28). 'PTC-HFpEF' and 'Global' congestion clusters had predominant HFpEF subtypes and similar proportions of NYHA class III/IV at baseline. However, patients in the 'Global' congestion phenotype were older (mean age 83 vs. 79) and had higher left heart volumes, higher filling pressure and lower TAPSE compared to the 'PTC-HFpEF' phenotype. A similar distinction was also observed in patients with HFpEF when unsupervised machine learning analysis was conducted using demographic and echocardiography

markers (29) emphasizing the role of congestion markers in distinguishing these phenotypes. Moreover, the 'Global' congestion phenotype incurred higher risk even after adjusting for natriuretic peptide levels in both the Nancy-HF and BIOSTAT-CHF cohorts, thus confirming that within HFpEF phenogroups with similar metabolic comorbidities, congestion phenotypes could also influence prognosis. It is possible that these two groups require distinct management strategies to improve congestion and overall prognosis. Our findings align with a previous study which identified HF clusters using clinical congestion variables such as JVD, leg edema, S3, crackles, and orthopnea in AHF (30). In effect, using clustering analysis on physical signs of congestion, Nimmi et al. found that the cluster primarily characterized by congestion with JVD was associated with a higher risk of adverse outcome compared to clusters with no signs of congestion (30). However, 'PTC, STC -AF' was associated herein with increased risk in the Nancy-HF cohort but not in the BIOSTAT-CHF cohort, probably because the validation cohort had relatively preserved left atrial volume and E/A ratio despite their similarity in terms of age and other characteristics such as eGFR, SBP and LVEF.

The biological background of congestion phenotypes

The differences observed in the five congestion phenotypes were reflected in the network analysis of proteins associated with each individual phenotype. 'PTC-HFpEF' was significantly associated with DDX58 (retinoic acid-inducible gene I), REN (Renin) and DECR1 (2,4-dienoyl-CoA reductase) proteins, which were downregulated compared to the 'PTC-dilated LV' phenotype. REN is an established biomarker having prognostic significance in heart failure whereas DDX58 and DECR1 are relatively new proteins identified in the present study in association with congestion and HF. DDX58 is central in regulating immune system pathways (31) while DECR1 plays a key role in fatty acid metabolism in myocardial cells (32). A previous mechanistic study in mice demonstrated that excessive fatty acids reduced DDX58 protein expression, thereby attenuating the autophagic

responses required for clearing toxic lipid inclusion bodies which may lead to inflammation-mediated cell death. (33)

In the ‘PTC, STC-AF’ phenotype, FURIN protein was linked with pathways related to the immune system, kinase-mediated signaling and extracellular matrix organization. FURIN, a pro convertase protein, converts NTproBNP to active forms, cleaves pro-protein PCSK 9 and plays a role in blood pressure regulation and atherosclerotic plaque formation (34). Moreover, an experimental study demonstrated that FURIN overexpression was associated with atrial remodeling in early HF (35). FURIN could hence be a potential biomarker for identifying patients with early AF and HF given its role in atrial remodeling and overall early development in atrio-ventricular junctions (36). Dysregulated ITGA6 and MPO, which are also involved in pathways linked to the ‘PTC, STC-AF’ phenotype, are associated with increased cardiac fibrosis and pressure overload as well as systolic and diastolic dysfunction in HF (37-39).

‘PIVC-dilated LA and LV’ was associated with kinase-mediated signaling, TNF-regulated cell death, hemostasis, immune system and vesicle-mediated transport. Network analysis showed that ERBB2 signaling, FASLG (member of the TNF superfamily) and TRAF2 (member of the TNF receptor associated factor) were focal points of the network. Our findings partially align with previous studies, which identified HFrEF as more prominently associated with pathways of kinase-mediated signaling, TNF-mediated cell response (40, 41) and hemostatsis (42). Dysregulation of EGFR:erbB2 axis (epidermal growth factor receptor and EGFR tyrosine kinase) is associated with HF severity and regulates apoptosis and myocyte hypertrophy (43, 44). It plays an important role in the myocardial response to stress overload and may represent a potential therapeutic target (43, 45). Remarkably, the ‘Global’ congestion phenotype was solely associated with Gal-4 protein only, indicating a large overlap of dysregulated proteins with other phenotypes. Gal-4 was not associated with any particular biological pathway in the Reactome. Gal-4, a transporter of apical proteins such as protease dipeptidyl peptidase-4 (DPP4) (46), has been shown to be linked with an increased risk

of incident HF (47) as well as increased risk of adverse events in severe HF (48) and HF with obesity and diabetes in recent studies (46). The other members of the galectin family are associated with the inflammatory immune response in cardiovascular metabolic diseases; however, studies on Gal-4 are limited and further research is warranted to elucidate its role in fibrosis and congestion in HF.

While immune system dysregulation was a recurring theme across all phenotypes, both the associated proteins and underlying pathways, on the other hand, varied according to phenotype. Although inflammation and immune responses have been associated with cardiovascular diseases for the past three decades, targeting specific immune molecules have failed to show favorable outcomes to date (49). The present findings further emphasize the heterogeneity in immune response depending on HF subtype, comorbidities and type of congestion, and that each phenotype may require a more personalized treatment approach. The network analysis performed herein enabled providing meaningful insight into the key mechanisms underpinning congestion phenotypes of HF. A greater understanding of these pathways could prove highly useful in potentially identifying diagnostic and therapeutic targets.

Assigning clusters to identified phenotypes using the random forest model and validation

While cluster assignment appears clinically intuitive based on differences in clinical characteristics, particularly given the overlap of clinical features, an AI-based model may better assign phenotypes by analyzing the grouping of several clinical and echocardiographic characteristics that may not be possible clinically. Our RF model exhibited 77% accuracy in predicting the cluster sub-group. It should be noted that the accuracy of the RF model in assigning clusters was much higher for the ‘PTC-dilated LV’ and ‘PTC, STC -AF’ phenotypes while lower for the ‘Global’ congestion phenotype. The usefulness of this RF model was moreover validated in the BIOSTAT-CHF cohort, thus confirming the prognostic value of using the RF model for patient cluster prediction which can be accessed through the (Ai-Cong-HF) webpage available to all

clinicians during their consultations, (https://cic-p-nancy.fr/ai-cong-hf/). As this model requires few clinical and echocardiographic variables, the model can easily be used in clinics or in future studies to study the different AHF phenotypes.

In addition to RF model, we explored XGBoost as AI-model to predict clusters. XGBoost had similar prediction performance as RF model (F1 measure ~ 78%). However, in this analysis, the characteristics of the predicted phenotypes in the validation dataset were not congruent with the characteristics of the derivation cohort when XGBoost model was used. Therefore, the RF models presented in this study offer superior validity. This is in line with published data suggesting that RF models are generally more robust against overfitting compared to XGBoost models. (50)

Clinical implications of our findings

These findings confirm that patients may be sub-classified into similar congestion profiles within a very heterogeneous AHF population. While most previous studies conducted unsupervised clustering to identify AHF phenotypes, the present model used only variables contributing to congestion assessment to identify congestion phenotypes, thereby contributing toward a better understanding of clinical and cardiac structural and functional differences associated with different congestion phenotypes in patients with AHF. Among the identified proteins and pathways, several well-documented pathways and proteins were found including REN, MPO and members of TNF and associated families, but also relatively new, less established proteins in the HF domain such as DDX58, FURIN, ERBB2 and Gal-4. These protein pathways provide clues for designing more innovative clinical studies assessing novel HF therapies. Furthermore, clinicians may use the weblink to assess their patients' congestion profile and prognosis. This tool offers a preliminary framework that permits labelling patients in a routine clinical setting and which may, in the future, contribute towards overall clinical management of these patients, as previously done. (51) Whether

decongestive strategies should be adapted to the congestion profiles predicted by our model warrants investigation in subsequent trials.

Limitations

This is a retrospective study, hence the findings must be considered as hypothesis-generating. Missing data could have impacted the cluster designation and RF model. The treating physician assessed the clinical congestion signs and echocardiographic parameters which may be biased due to inter-observer variability. Data on inferior vena cava diameter were not available and thus intravascular congestion status based on JVD could not be reliably assessed. In addition, the Nancy-HF cohort consists of single-center patient data, with a homogenous patient population with regard to demographic characteristics and access to healthcare. However, validation in a large multicenter European cohort (BIOSTAT-CHF) addresses this limitation to some degree. While the results were validated in another European cohort, these findings may not be valid for other populations. Although E/e', TAPSE and PAPS were not in the top 5 discriminants of the RF model, their unavailability in the BIOSTAT-CHF cohort could have impacted the cluster classification to some extent. In addition, the two populations had different demographic profiles (age of patients, proportion of women) which could be a reason for the observed differences) in the risk associated with 'PTC, STC-AF' and 'PIVC-dilated LA and LV' phenogroups. We could not compare the classification done using clustering analysis with that of Forrester classification because data on perfusion status was not collected in the Nancy-HF cohort. Furthermore, some of the prognostic markers such as hypertrophic cardiomyopathy were not considered in the clustering analysis because of limited data availability. Furthermore, some of the prognostic markers such as hypertrophic cardiomyopathy were not considered in the clustering analysis because of limited data availability. However, given the relative rarity of hypertrophic cardiomyopathy and the inclusion of LVMi in the clustering process, we believe this limitation is unlikely to have a significant impact on the results. Also, Cox proportional hazard models could not be adjusted for ARNi and SGLT2i as the data was

collected between 2015 and 2019 when ARNi were prescribed in 3% population and SGLT2i were available in France after April 2020.

Conclusion

In patients with AHF, clustering techniques identified five clinical congestion profiles associated with both long-term clinical risk and differences in biomarkers, suggesting potential different underlying pathophysiologies. Phenotyping patients according to their congestion profile could prove useful in further studies assessing novel therapeutic approaches in AHF.

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Data availability statement:

Data that supports the findings of this study are available on the reasonable request to authors and with permission of the sponsors.

Ethical approval

NANCY-HF is a single-centre study comprised data gathered from hospital charts and, per French law, was declared to the National Commission for Information Technology and Civil Liberties (CNIL) and did not require individual patient consent.

BIOSTAT-CHF was approved by the local medical ethical committee at each center and complied with the Declaration of Helsinki. All participants provided written informed consent prior to any study handling.

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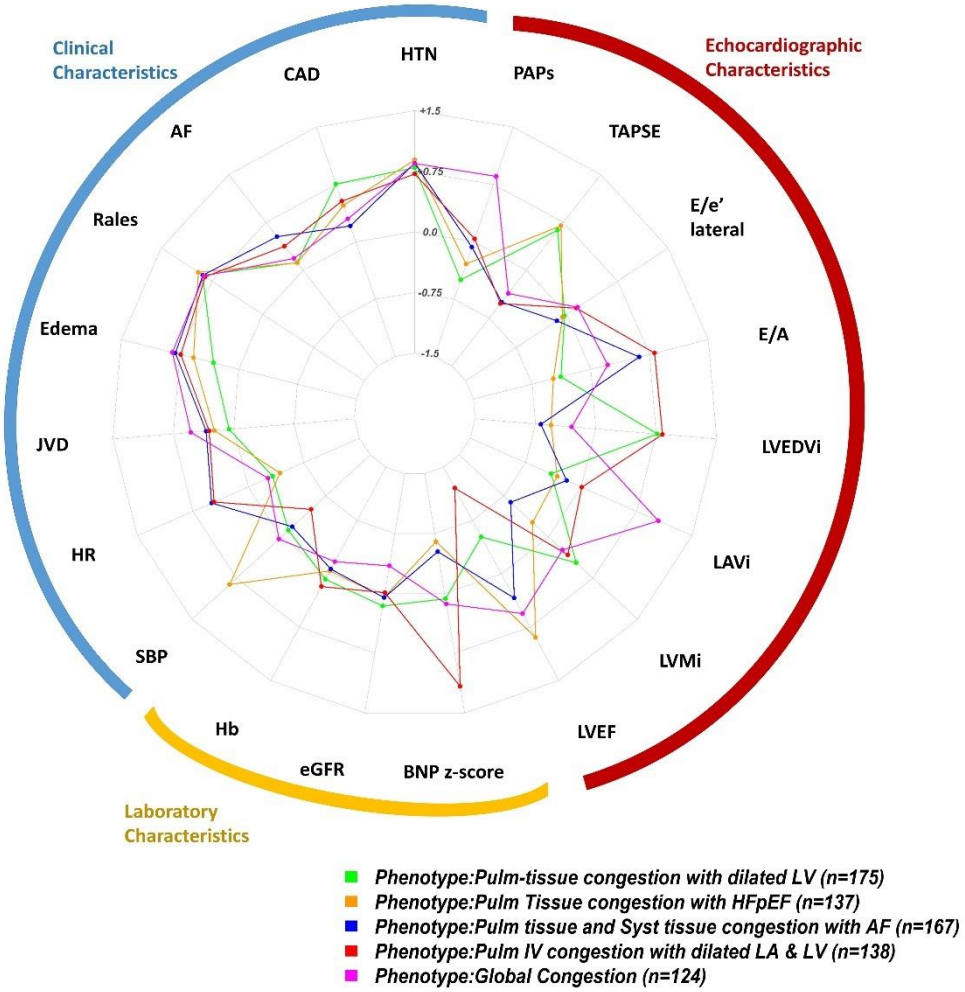
Figure legends

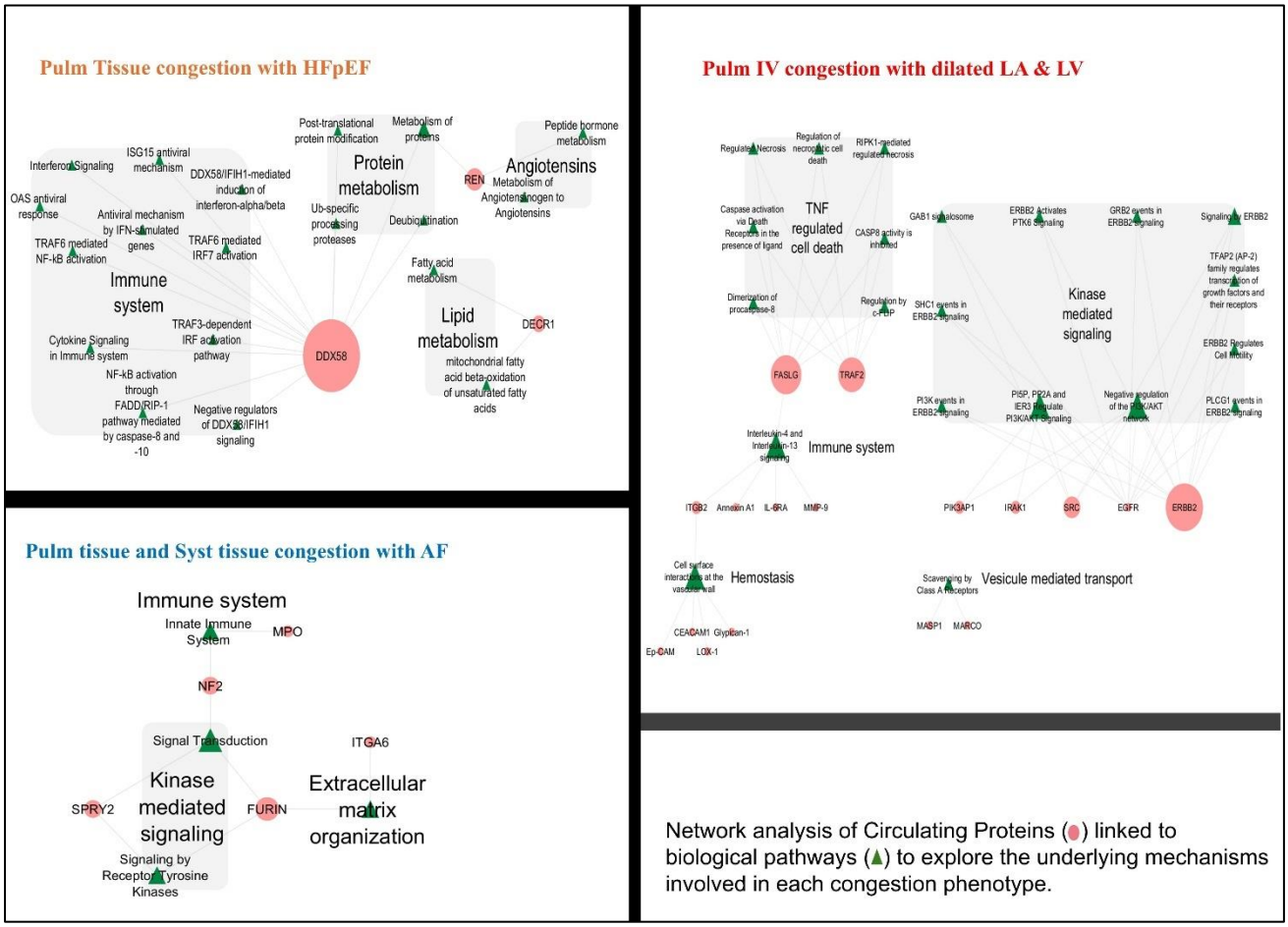
Figure 1: Radar plot depicting the clustering variables in Nancy-HF



Legend: The axes represent the different congestion markers. The congestion phenotype is represented by polygons, with points on each axis corresponding to the standardized mean value of the respective congestion marker for that phenotype.

Abbreviations: CAD: Coronary Artery Disease; AF: Atrial Fibrillation; HTN: hypertension; HR: Heart Rate; SBP: Systolic Blood Pressure; JVD: Jugular Venous Distention; Hb: Hemoglobin; eGFR: estimated Glomerular Filtration Rate; BNP: Brain Natriuretic Peptide; LVEF: Left Ventricular Ejection Fraction; LVMi: Left Ventricular Mass index;; LAVi: Left Atrial Volume index; LVEDVi: Left Ventricular End Diastolic Volume index; TAPSE: Tricuspid Annular Plane Systolic Excursion; PASP: Pulmonary artery systolic pressure

Figure 2: Biological pathways linked to protein biomarkers significantly associated with each phenotype in BIOSTAT-CHF



Legend: The size of the protein node (pink circles) corresponds to the number of connected pathways with the respective protein while the size of the pathway node (green triangles) corresponds to the number of proteins linked to the pathway.
All pathways linked to the identified proteins are presented for ‘Pulm Tissue congestion with HFpEF’. Pathways connecting at least 2 proteins are presented for ‘Pulm tissue and Syst tissue congestion with AF’. Over-represented pathways are presented for ‘Pulm IV congestion with dilated LA & LV’.

Figure 3a: Kaplan-Meier curves according to identified phenotype in Nancy-HF

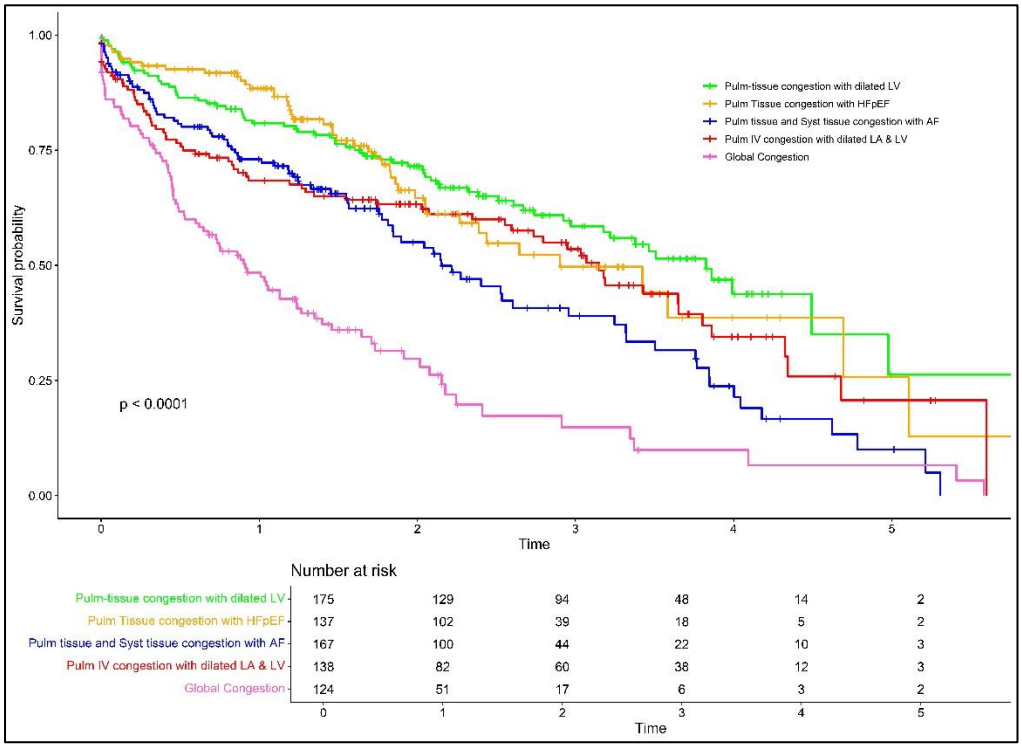
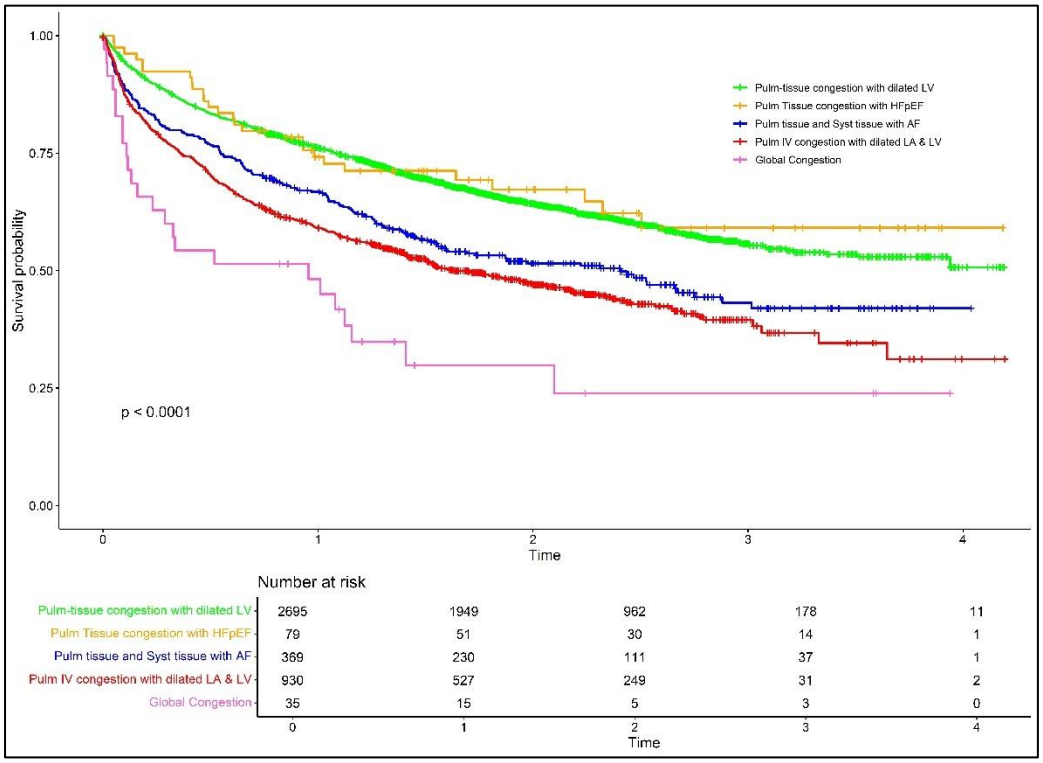


Figure 3b: Kaplan-Meier curves according to predicted phenotype in BIOSTAT-CHF.



The outcome was a composite of hospitalization for HF or death in the derivation cohort and the validation cohort (BIOSTAT-CHF).

Graphical abstract

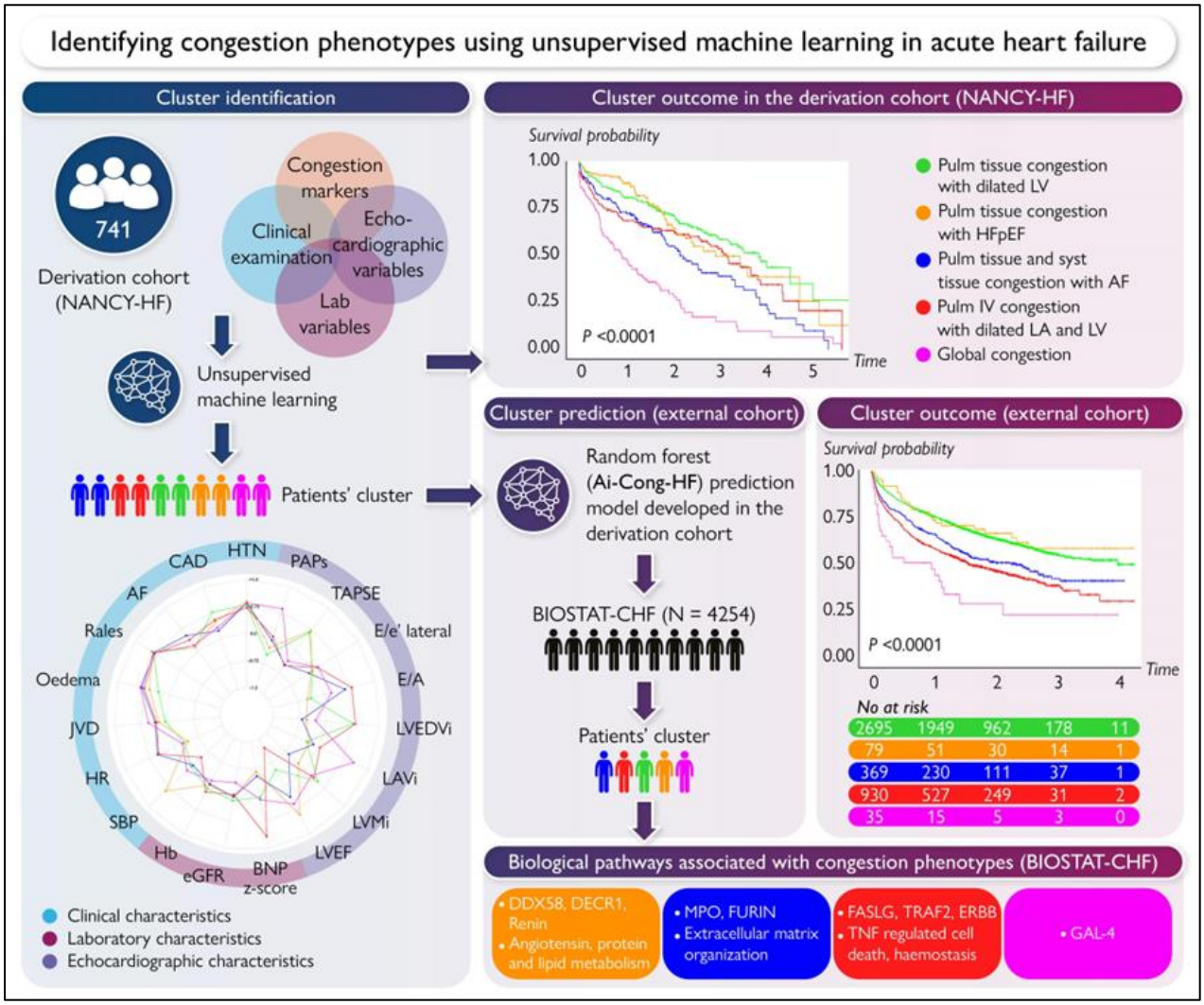


Table 1: Baseline patient characteristics according to the identified phenotype in the Nancy-HF cohort.

Characteristic		Pulm-tissue congestion with dilated LV	Pulm tissue congestion with HFpEF	Pulm tissue and Syst tissue congestion with AF	Pulm IV congestion with dilated LA & LV	Global congestion	p-value
Pulm tissue		+ + +	+ + +	+ + +	+ +	+ + +	
Syst tissue		+	+ +	+ + +	+ +	+ + +	
Syst – IV		+	+ +	+ +	+ +	+ + +	
Pulm – IV		+	+	++	+ + +	+ + +	
	N	**N = 175**	**N = 137**	**N = 167**	**N = 138**	**N = 124**	
Age	741	74 (66, 83)	79 (71, 85)	79 (70, 87)	73 (64, 81)	83 (74, 89)	<0.001
sex = female	741	77 (44%)	71 (52%)	111 (66%)	51 (37%)	63 (51%)	<0.001
BMI	712	26 (5.4)	28.7 (6.7)	28.8 (.3)	26.6 (5.1)	25.5 (5.5)	<0.001
Hypertension	741	139 (79%)	122 (89%)	142 (85%)	99 (72%)	105 (85%)	0.002
CAD	640	102 (76%)	55 (47%)	32 (21%)	61 (53%)	36 (30%)	<0.001
AF	740	21 (12%)	16 (12%)	88 (53%)	52 (38%)	23 (19%)	<0.001
Diabetes	741	66 (38%)	70 (51%)	54 (32%)	43 (31%)	45 (36%)	0.004
Smoking	741	59 (34%)	46 (34%)	45 (27%)	47 (34%)	33 (27%)	0.4
COPD	499	12 (19%)	27 (26%)	28 (19%)	7 (9.6%)	16 (14%)	0.044
HR	708	72 (14)	70 (13)	91 (23)	90 (33)	73 (16)	<0.001
SBP	740	134 (28)	166 (33)	132 (22)	122 (28)	139 (32)	<0.001
NYHA	741						0.91
Class I		3 (1.7%)	0 (0%)	0 (0%)	2 (1.4%)	0 (0%)	
Class II		16 (9.1%)	7 (5.1%)	8 (4.8%)	12 (8.7%)	5 (4.0%)	
Class III		58 (33%)	60 (44%)	74 (44%)	64 (46%)	54 (44%)	
Class IV		98 (56%)	70 (51%)	85 (51%)	60 (43%)	65 (52%)	
Rales	499	55 (89%)	97 (95%)	131 (89%)	62 (85%)	99 (87%)	0.22
Crackles lower lung zones	499	47 (76%)	94 (92%)	109 (74%)	47 (64%)	91 (80%)	<0.001
Crackles lower-upper lung zones	499	29 (47%)	51 (50%)	52 (35%)	20 (27%)	47 (41%)	0.019
Wheezing	499	19 (31%)	31 (30%)	43 (29%)	11 (15%)	18 (16%)	0.011
Peripheral edema	741	56 (32%)	79 (58%)	135 (81%)	102 (74%)	105 (85%)	<0.001
JVD	741	10 (5.7%)	33 (24%)	57 (34%)	42 (30%)	66 (53%)	<0.001
LVEF	702	41 (12)	63 (7)	54 (11)	30 (12)	58 (9)	<0.001
HF type	702						<0.001
HFmrEF		46 (30%)	2 (1.5%)	36 (23%)	20 (15%)	18 (15%)	
HFpEF		35 (23%)	130 (98%)	106 (67%)	8 (6.0%)	103 (84%)	
HFrEF		74 (48%)	0 (0%)	17 (11%)	105 (79%)	2 (1.6%)	
Hb	732	12.43 (2.18)	12.17 (2.07)	12.11 (2.18)	12.65 (2.37)	11.88 (2.09)	0.053
eGFR	730	59 (25)	56 (25)	57 (24)	55 (25)	47 (22)	<0.001
BNP at admission	656	1,028 (610, 1,556)	420 (271, 568)	521 (324, 746)	2,002 (1,206, 3,226)	1,041 (613, 1,686)	<0.001
LVMi	671	136 (55)	104 (32)	89 (23)	130 (38)	126 (42)	<0.001
E/A	431	1.04 (0.71, 1.59)	1.03 (0.75, 1.39)	2.16 (1.62, 2.48)	2.10 (1.37, 2.92)	1.61 (1.07, 2.44)	<0.001
e' prime velocity	320	6.30 (1.98)	7.20 (2.52)	8.57 (2.12)	6.54 (2.21)	7.25 (2.15)	<0.001
E/e' mean	319	13.8 (5.8)	14.1 (6.5)	12.6 (4.9)	15.2 (6.2)	15.8 (5.2)	0.004
E/e' lateral	624	11.0 (9.0, 14.9)	11.1 (8.6, 15.1)	11.1 (8.3, 13.7)	12.7 (9.7, 16.5)	12.4 (9.9, 17.2)	0.014
LAVi	669	38 (14)	41 (18)	44 (15)	50 (20)	80 (46)	<0.001
LVEDVi	663	78 (27)	41 (14)	37 (12)	79 (29)	48 (19)	<0.001
TAPSE	690	21.3 (4.4)	21.6 (4.1)	15.6 (3.8)	15.5 (4.3)	16.3 (4.2)	<0.001
PASP	630	37 (11)	40 (15)	44 (13)	45 (11)	58 (19)	<0.001
Diuretics	728	124 (72%)	103 (76%)	147 (89%)	118 (88%)	114 (94%)	<0.001
ARNi	728	8 (4.6%)	1 (0.7%)	1 (0.6%)	10 (7.4%)	2 (1.7%)	0.002
MRA	728	43 (25%)	22 (16%)	25 (15%)	37 (27%)	30 (25%)	0.031
Beta-blockers	729	147 (85%)	99 (73%)	133 (81%)	115 (85%)	91 (75%)	0.025
ACEi	729	110 (63%)	68 (50%)	73 (45%)	79 (59%)	56 (46%)	0.003
ARA	728	28 (16%)	43 (32%)	40 (24%)	14 (10%)	22 (18%)	<0.001

Pulm: Pulmonary; LA: Left Atrium; LV: Left Ventricle; BMI: Body Mass Index; CAD: Coronary Artery Disease; AF: Atrial Fibrillation; COPD: Chronic Obstructive Pulmonary Disease; HR: Heart Rate; SBP: Systolic Blood Pressure; NYHA: New York Heart Association; JVD: Jugular Venous Distention; HF: heart failure; HFmrEF: HF with mildly-reduced Ejection Fraction; HFpEF: HF with preserved Ejection Fraction; HF with reduced Ejection Fraction; Hb: Hemoglobin; eGFR: estimated Glomerular Filtration Rate; BNP: Brain Natriuretic Peptide; LVEF: Left Ventricular Ejection Fraction; LVMi: Left Ventricular Mass index;; LAVi: Left Atrial Volume index; LVEDVi: Left Ventricular End Diastolic Volume index; TAPSE: Tricuspid Annular Plane Systolic Excursion; PASP: Pulmonary artery systolic pressure; ACEi: Angiotensin Converting Enzyme inhibitor; ARA: Angiotensin Receptor Antagonist; ARNi: Angiotensin and Neprilysin Inhibitor: MRA: Mineralocorticoid Receptor Antagonist

Categorical variables presented as n(%), continuous variables as mean(SD) if distribution normal or Median (Q1, Q3) if distribution non normal.

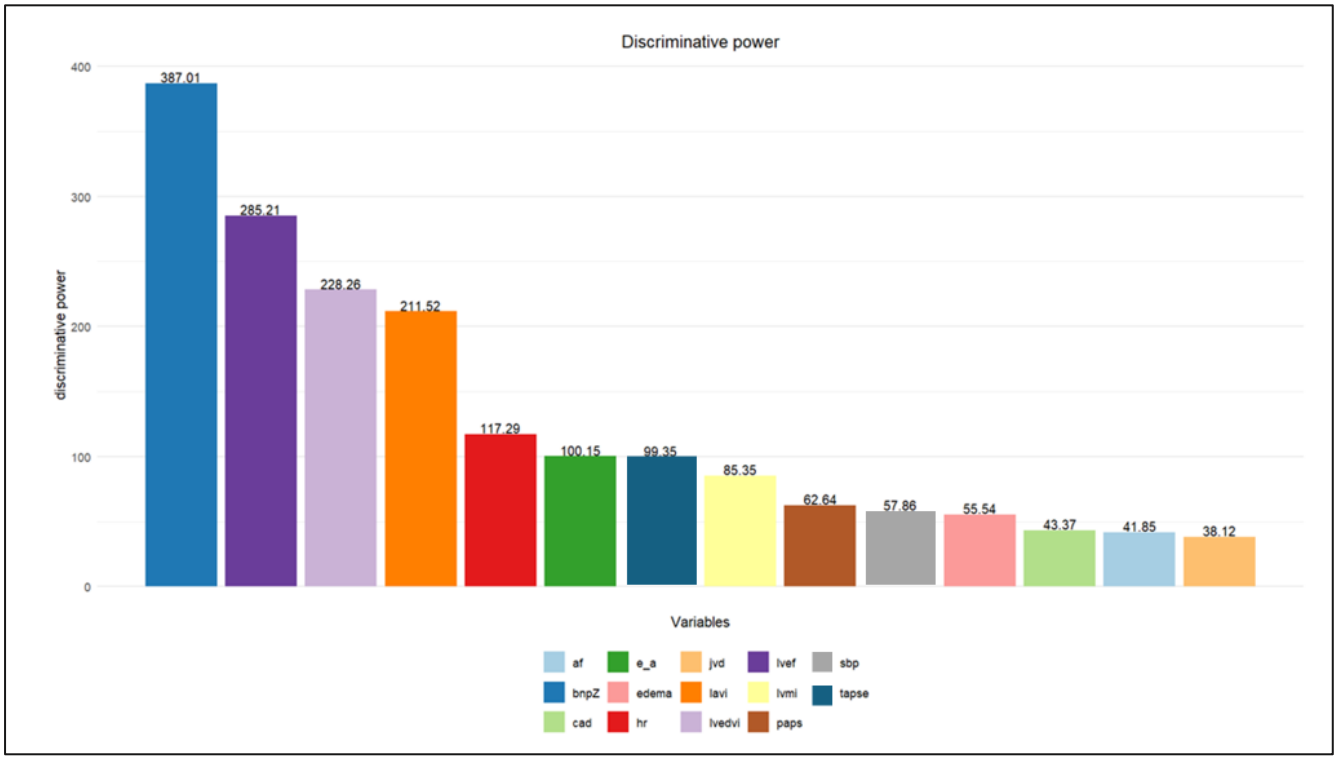
Table 2: Association of identified phenotype with composite outcome in the Nancy-HF and BIOSTAT-CHF cohorts

Phenotype	N events /N	Nancy-HF HR (95% CI)	p-value	N events /N	BIOSTAT-CHF HR (95% CI)	p-value
Unadjusted	361/741			1702 /4109		
Pulm-tissue congestion with dilated LV		Ref.			Ref.	
Pulm Tissue congestion with HFpEF		1.07 (0.74 - 1.55)	0.73		0.91 (0.62 - 1.34)	0.64
Pulm tissue and Syst tissue congestion with AF		1.80 (1.31 - 2.48)	0.0003		1.49 (1.27 - 1.74)	<0.0001
Pulm IV congestion with dilated LA & LV		1.40 (1.01 - 1.96)	0.045		1.76 (1.58 - 1.96)	<0.0001
Global congestion		3.52 (2.56 - 4.85)	<0.0001		2.96 (1.98 - 4.45)	<0.0001
Model 1*	330/668			1519/3705		
Pulm-tissue congestion with dilated LV		Ref.			Ref.	
Pulm Tissue congestion with HFpEF		0.98 (0.60 - 1.59)	0.93		0.78 (0.49 - 1.24)	0.29
Pulm tissue and Syst tissue congestion with AF		1.50 (1.01 - 2.23)	0.044		1.13 (0.92 - 1.41)	0.25
Pulm IV congestion with dilated LA & LV		1.37 (0.94 - 2.00)	0.099		1.65 (1.46 - 1.87)	<0.0001
Global congestion		2.38 (1.61 - 3.52)	<0.0001		1.82 (1.17 - 2.83)	0.008
Model 2 *	303/605			1082/2455		
Pulm-tissue congestion with dilated LV		Ref.			Ref.	
Pulm Tissue congestion with HFpEF		1.16 (0.68 - 1.98)	0.58		0.74 (0.45 - 1.20)	0.22
Pulm tissue and Syst tissue congestion with AF		1.74 (1.13 - 2.67)	0.012		1.05 (0.83 - 1.32)	0.69
Pulm IV congestion with dilated LA & LV		1.23 (0.81 - 1.86)	0.33		1.43 (1.21 - 1.70)	<0.0001
Global congestion		2.41 (1.60 - 3.63)	<0.0001		1.64 (1.04 - 2.58)	0.033

Reference: Pulm-tissue congestion with dilated LV
Model 1: adjusted for age, sex, BMI, SBP, LVEF, eGFR.
Model 2: Model M1 + NT-proBNP z score
The outcome was a composite of re-hospitalization for HF or death in the derivation cohort (Nancy-HF) and a composite of death and hospitalization i
validation cohort (BIOSTAT-CHF).

Supplementary data

Figure 1S: Plot showing discriminative power of variables in the LCM model



**R plot can fill a maximum of 12 colors. Hence, colors for SBP and TAPSE were added manually.*

Figure 2S: performance characteristics of the random forest model

Random forest: iterations 250

Classifier output

```
=== Stratified cross-validation ===
=== Summary ===

Correctly Classified Instances         573               77.3279 %
Incorrectly Classified Instances       168               22.6721 %
Kappa statistic                          0.7131
Mean absolute error                      0.1961
Root mean squared error                  0.2792
Relative absolute error                 61.5414 %
Root relative squared error             69.9429 %
Total Number of Instances              741

=== Detailed Accuracy By Class ===

                 TP Rate  FP Rate  Precision  Recall   F-Measure  MCC      ROC Area  PRC Area  Class
                 0.931    0.136    0.679      0.931    0.786      0.722    0.962     0.893     1
                 0.638    0.017    0.898      0.638    0.746      0.714    0.971     0.901     2
                 0.826    0.077    0.758      0.826    0.791      0.728    0.962     0.895     3
                 0.818    0.045    0.806      0.818    0.812      0.769    0.975     0.903     4
                 0.581    0.016    0.878      0.581    0.699      0.672    0.963     0.837     5
Weighted Avg.    0.773    0.063    0.794      0.773    0.770      0.722    0.966     0.887

=== Confusion Matrix ===

   a   b   c   d   e   <-- classified as
 163   5   1   6   0 |   a = 1
  37  88   8   0   5 |   b = 2
  11   2 138  11   5 |   c = 3
   8   0  17 112   0 |   d = 4
  21   3  18  10  72 |   e = 5
```

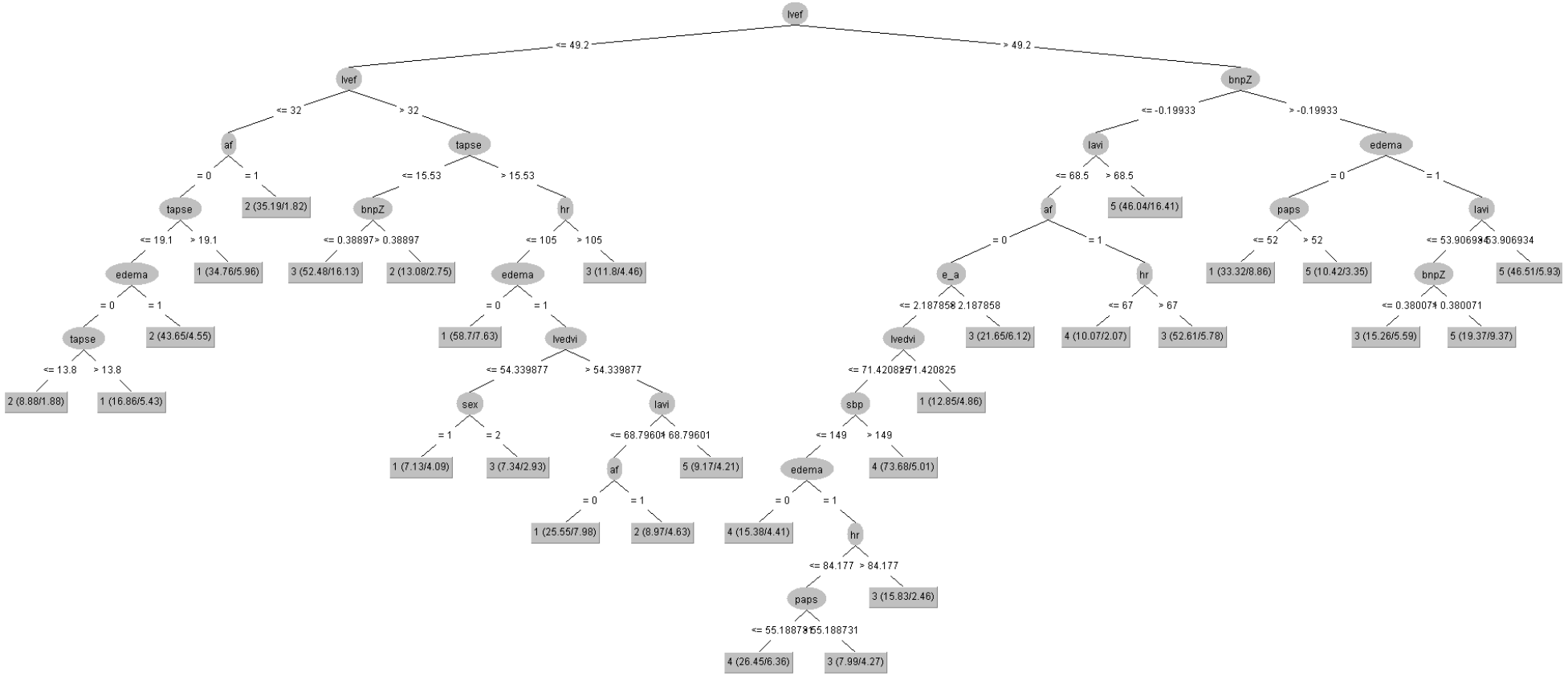
```
weka.classifiers.trees.RandomTree -K 0 -M 1.0 -V 0.001 -S 1 -do-not-check-capabilities

Attribute importance based on average impurity decrease (and number of nodes using that attribute)

      0.39 (  3727)  lvef
      0.36 (  2354)  lvedvi
      0.36 (  2753)  lavi
      0.35 (  2440)  lvmi
      0.35 (   115)  rales
      0.34 (  3685)  hr
      0.34 (  3098)  tapse
      0.3  (  2174)  paps
      0.3  (  1689)  e_eplat
      0.25 (  6528)  bnpZ
      0.25 (  1666)  e_a
      0.24 (  7912)  sbp
      0.23 (  6586)  bmi
      0.23 (  8499)  age
      0.23 (  1697)  cad
      0.22 (  7384)  hb
      0.22 (  2297)  jvd
      0.22 (  7178)  egfr
      0.21 (  2501)  af
      0.18 (  3382)  edema
      0.18 (  2862)  sex
      0.17 (  1529)  htn
```

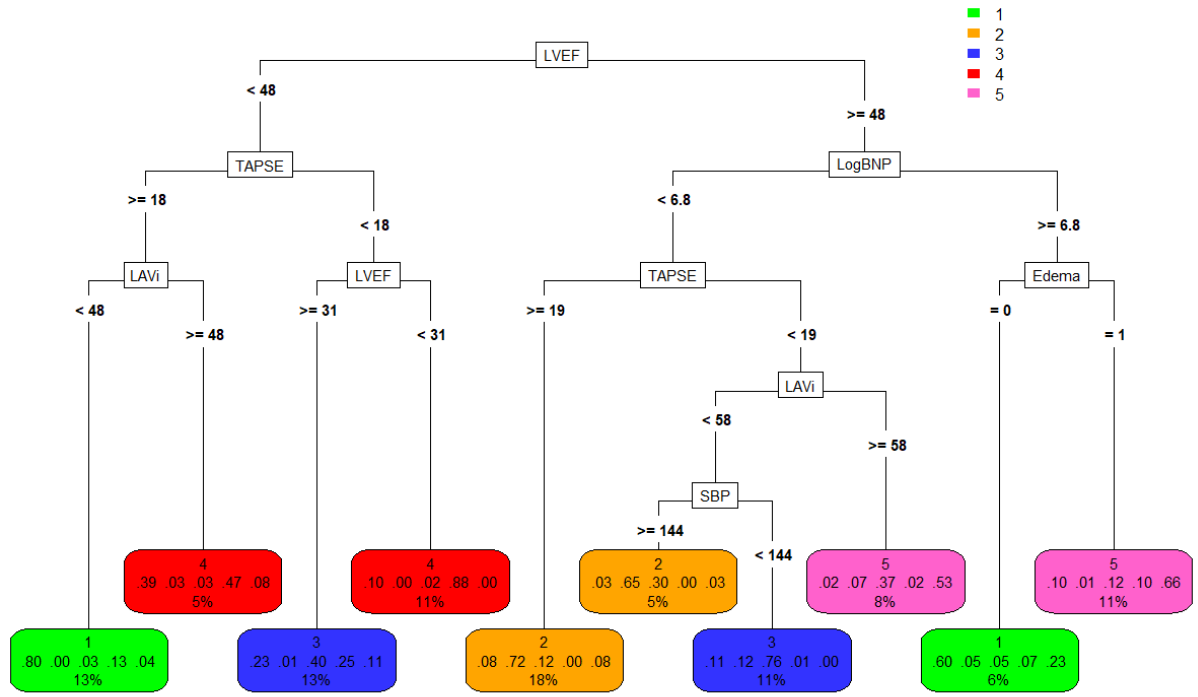
Decision Tree:

Figure 3S: Detailed decision tree with minimal instances per leaf =7



The first number in brackets corresponds to the total number of classified objects and the 2nd number corresponds to misclassified objects.

Figure 4S: Decision tree: simplified with logBNP

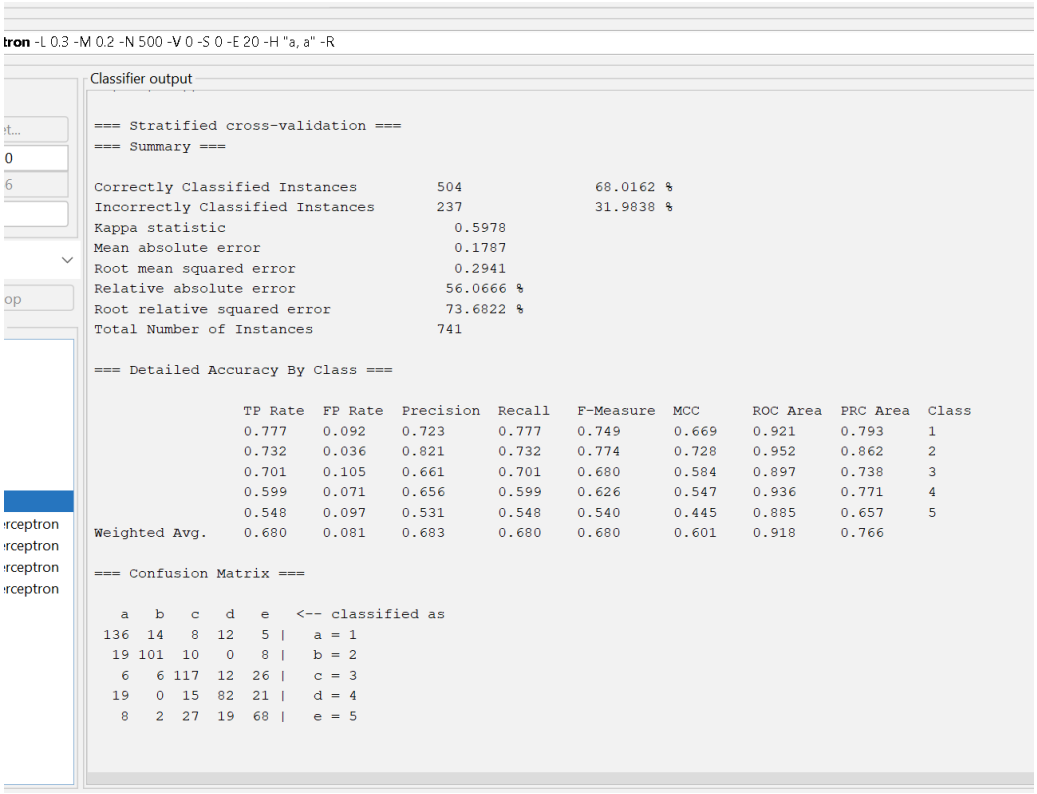


Simplified decision tree with log BNP instead of BNP z-score.

The number corresponding to the cluster number represents the correctly classified patients in the leaf. The reported percentages represent the percentage of patients in each leaf.

For example, in cluster 1 (green box): 80 % patients were of cluster 1, 0% patients were of cluster 2, 3% patients were of cluster 3, 13% patients were of cluster 4 and 4% patients were of cluster 5. A total of 13% patients from the dataset were classified as cluster 1 (sum may not be 100% due to rounding).

Figure 5S: performance characteristics of the support vector machine model



tron -L 0.3 -M 0.2 -N 500 -V 0 -S 0 -E 20 -H "a, a" -R

Classifier output

```
=== Stratified cross-validation ===
=== Summary ===

Correctly Classified Instances         504               68.0162 %
Incorrectly Classified Instances       237               31.9838 %
Kappa statistic                          0.5978
Mean absolute error                      0.1787
Root mean squared error                  0.2941
Relative absolute error                 56.0666 %
Root relative squared error             73.6822 %
Total Number of Instances              741

=== Detailed Accuracy By Class ===

               TP Rate   FP Rate   Precision   Recall  F-Measure  MCC      ROC Area  PRC Area  Class
               0.777     0.092     0.723      0.777     0.749      0.669     0.921     0.793     1
               0.732     0.036     0.821      0.732     0.774      0.728     0.952     0.862     2
               0.701     0.105     0.661      0.701     0.680      0.584     0.897     0.738     3
               0.599     0.071     0.656      0.599     0.626      0.547     0.936     0.771     4
               0.548     0.097     0.531      0.548     0.540      0.445     0.885     0.657     5
Weighted Avg.  0.680     0.081     0.683      0.680     0.680      0.601     0.918     0.766

=== Confusion Matrix ===

   a   b   c   d   e   <-- classified as
 136  14   8  12   5 |   a = 1
  19 101  10   0   8 |   b = 2
   6   6 117  12  26 |   c = 3
  19   0  15  82  21 |   d = 4
   8   2  27  19  68 |   e = 5
```

Table 1S: Baseline characteristics of phenotypes identified in BIOSTAT-CHF using the RF model

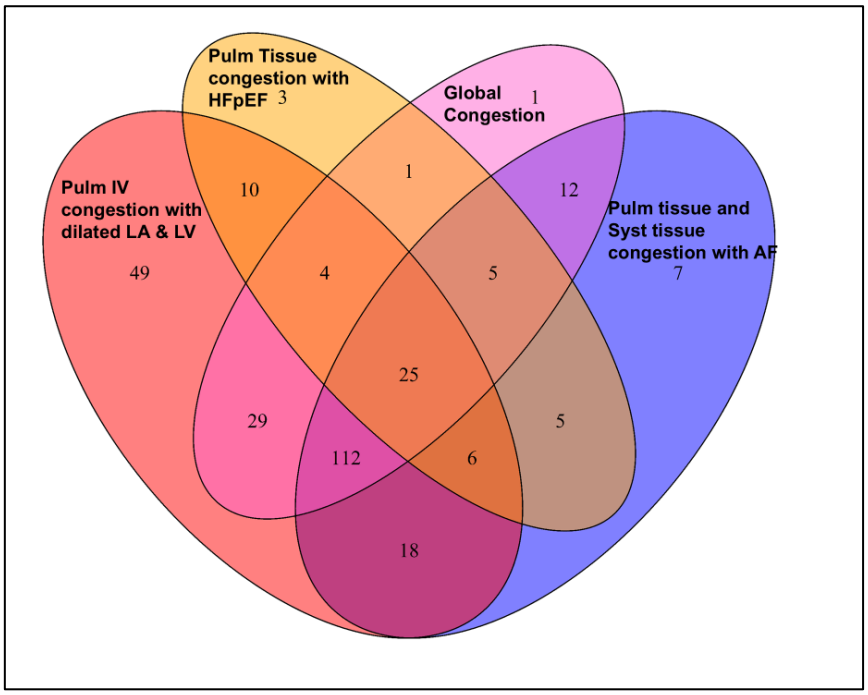
Characteristic		Pulm-tissue congestion with dilated LV	Pulm Tissue congestion with HFpEF	Pulm tissue and Syst tissue congestion with AF	Pulm IV congestion with dilated LA & LV	Global congestion	p-value [1]
	N	N = 2,796	N = 83	N = 384	N = 956	N = 35	
Age	4,254	71 (63, 78)	76 (69, 83)	78 (70, 83)	70 (62, 77)	84 (81, 88)	<0.001
Sex	4,254	738 (26%)	45 (54%)	250 (65%)	210 (22%)	20 (57%)	<0.001
BMI	4,181	28.2 (5.6)	30.3 (6.3)	30.7 (7.5)	27.8 (6.0)	28.2 (5.0)	<0.001
Hypertension	4,247	1,672 (60%)	73 (88%)	268 (70%)	533 (56%)	30 (86%)	<0.001
CAD	3,669	1,748 (73%)	57 (85%)	170 (59%)	496 (56%)	15 (65%)	<0.001
AF	4,240	990 (36%)	18 (22%)	267 (70%)	603 (63%)	25 (71%)	<0.001
Diabetes	4,245	878 (31%)	36 (43%)	128 (33%)	327 (34%)	11 (31%)	0.12
Smoking	4,239						
None		1,174 (42%)	38 (46%)	223 (59%)	375 (39%)	14 (42%)	
Past		1,220 (44%)	36 (43%)	124 (33%)	426 (45%)	19 (58%)	
Current		396 (14%)	9 (11%)	34 (8.9%)	151 (16%)	0 (0%)	
COPD	4,238	458 (16%)	13 (16%)	82 (22%)	194 (20%)	8 (23%)	0.016
NYHA	4,183						
I		58 (2.1%)	1 (1.2%)	4 (1.1%)	10 (1.1%)	0 (0%)	
II		1,236 (45%)	31 (38%)	98 (26%)	211 (23%)	4 (11%)	
III		1,218 (44%)	37 (46%)	198 (53%)	529 (56%)	18 (51%)	
IV		242 (8.8%)	12 (15%)	76 (20%)	187 (20%)	13 (37%)	
HR	4,209	73 (15)	69 (14)	86 (21)	88 (22)	73 (13)	<0.001
SBP	4,221	126 (21)	162 (29)	125 (19)	118 (21)	135 (29)	<0.001
Rales	4,099	1,121 (42%)	32 (41%)	231 (63%)	613 (66%)	29 (85%)	<0.001
Rales (type)	3,040						
None		1,211 (63%)	27 (71%)	112 (46%)	334 (41%)	15 (65%)	
Single Base		213 (11%)	3 (7.9%)	30 (12%)	113 (14%)	2 (8.7%)	
Bibasilar		501 (26%)	8 (21%)	100 (41%)	365 (45%)	6 (26%)	
Edema	3,643	1,013 (43%)	55 (73%)	321 (92%)	790 (93%)	32 (97%)	<0.001
JVD	3,230	352 (17%)	23 (35%)	144 (49%)	454 (62%)	31 (94%)	<0.001
Hb	3,989	13.29 (1.86)	13.02 (1.73)	12.51 (1.85)	13.12 (2.09)	11.75 (1.53)	<0.001
eGFR	4,248	66 (23)	62 (22)	60 (22)	58 (24)	48 (24)	<0.001
NT-proBNP	2,891	2,037 (708, 4,345)	697 (257, 1,780)	1,541 (710, 2,837)	8,314 (4,145, 14,234)	6,812 (4,956, 10,029)	<0.001
LVEF	3,811	35 (11)	59 (5)	54 (10)	26 (8)	59 (3)	<0.001
HF type	3,811						<0.001
HFmrEF		414 (16%)	3 (4.7%)	71 (25%)	28 (3.2%)	3 (9.1%)	
HFpEF		198 (7.8%)	61 (95%)	170 (60%)	2 (0.2%)	30 (91%)	
HFrEF		1,938 (76%)	0 (0%)	40 (14%)	853 (97%)	0 (0%)	
LVMi	2,397	144 (58)	122 (39)	105 (34)	145 (55)	115 (41)	<0.001
E/A	1,331	1.00 (0.70, 1.52)	0.90 (0.70, 1.30)	0.99 (0.70, 1.40)	2.27 (1.45, 3.00)	1.00 (0.83, 1.00)	<0.001
LAVi	2,942	27.2 (3.6)	25.6 (3.6)	26.9 (4.4)	28.6 (3.9)	27.4 (3.2)	<0.001
LVEDVi	3,369	86 (16)	67 (14)	62 (11)	90 (16)	65 (12)	<0.001

1 Kruskal-Wallis rank sum test; Pearson's Chi-squared test

Categorical variables presented as n(%), continuous variables as mean(SD) if distribution normal or Median (Q1, Q3) if distribution was non normal.

BMI: Body Mass Index; CAD: Coronary Artery Disease; AF: Atrial Fibrillation; COPD: Chronic Obstructive Pulmonary Disease; NYHA: New York Heart Association; HR: Heart Rate; SBP: Systolic Blood Pressure; JVD: Jugular Venous Distention; Hb: Hemoglobin; eGFR: estimated Glomerular Filtration Rate; BNP: Brain Natriuretic Peptide; LVEF: Left Ventricular Ejection Fraction; LVMi: Left Ventricular Mass index; LAVi: Left Atrial Volume index; LVEDVi: Left Ventricular End Diastolic Volume index

Supplementary Figure 6S: Venn diagram of protein biomarkers significantly associated with the different phenotypes in BIOSTAT-CHF



Phenotype	Total number of proteins associated with the phenotype	Biomarkers (OLINK Name)
Pulm Tissue congestion with HFpEF	59	REN, DECR1, DDX58
Pulm tissue and Syst tissue congestion with AF	190	MPO, ITGA6, NF2, SPRY2, ICA1, S100A4, FURIN
Pulm IV congestion with dilated LA & LV	253	ITGB2, MMP_9, IL_6RA, CHIT1, EP_CAM, PCSK9, EGFR, RARRES2, SRC, IDUA, LOX_1, PIgR, SERPINA12, AMBP, IL16, IgG_Fc_receptor_IIb, GDF_2, MARCO, MMP_12, TGM2, CASA, PARP_1, HAOX1, ZBTB16, TPSAB1, IRAK1, TRIM5, DCTN1, TRAF2, TRIM21, EGLN1, NFATC3, EIF4G1, HSD11B1, HEXIM1, BACH1, PIK3AP1, DFFA, MASP1, CEACAM1, GPC1, ERBB2, MetAP 2, FASLG, Gal-1, ERBB4, S100A11, ICOSLG, ANXA1
Global congestion	190	Gal_4

Supplementary Figure 7S: Proportion of outcome for each phenotype stratified by a) age, b) sex, and c) LVEF category.

Figure a

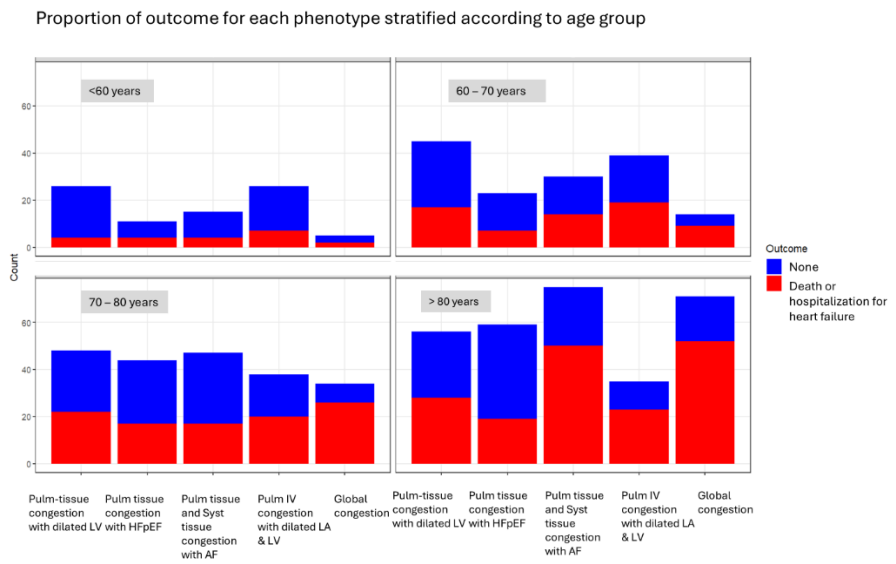


Figure b

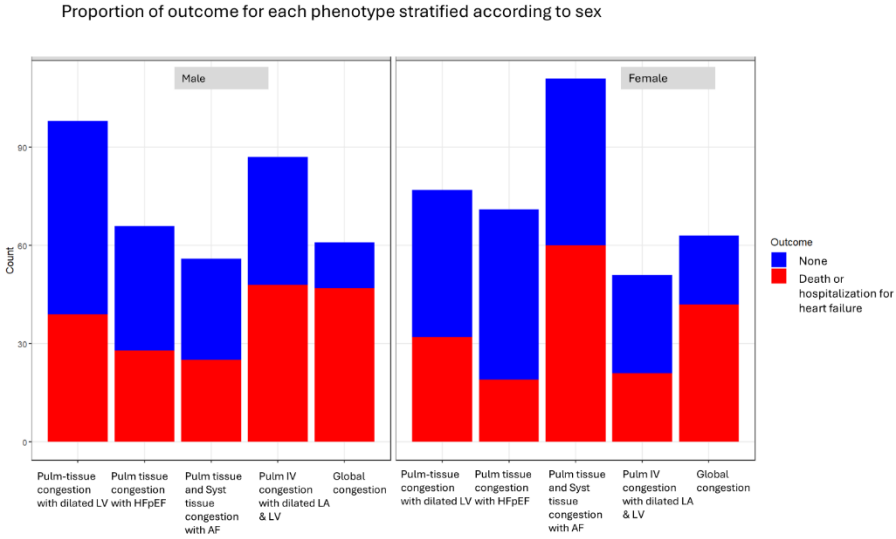


Figure c

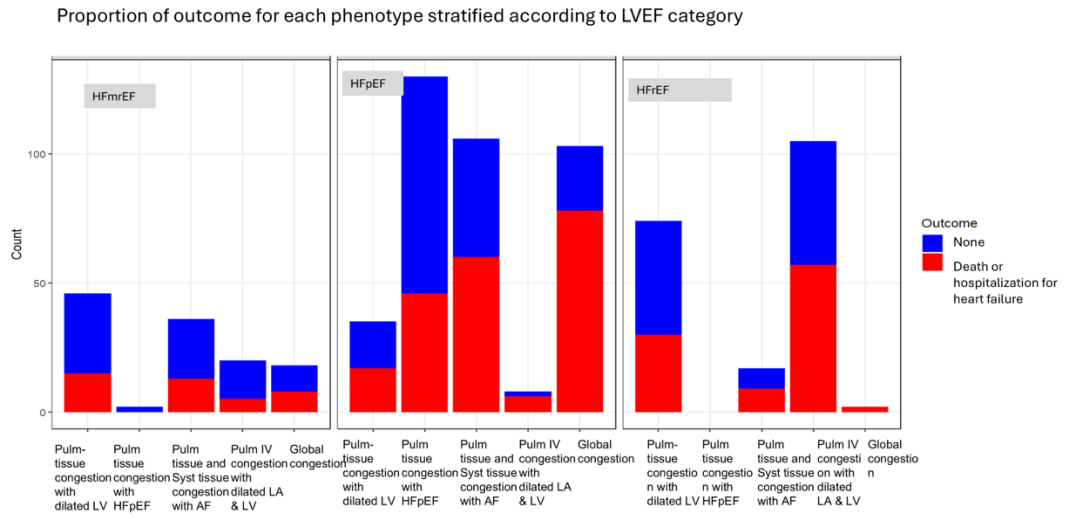


Table 2S: Association of protein biomarkers with congestion phenotype using the multinomial logistic regression model.

Biomarker	Pulm Tissue congestion with HFpEF			Pulm tissue and Syst tissue congestion with AF			Pulm IV congestion with dilated LA & LV			Global congestion			P overall	P-corrected
(Olink name)	OR	CI	p	OR	CI	p	OR	CI	p	OR	CI	p		
ITGB2	1.17	0.84, 1.62	0.3	1.02	0.87, 1.21	0.8	0.8	0.71, 0.89	<0.001	0.92	0.56, 1.52	0.8	<0.001	0.002
MMP_9	0.95	0.76, 1.17	0.6	0.96	0.86, 1.07	0.4	1.13	1.04, 1.22	0.002	0.9	0.65, 1.25	0.5	0.014	0.019
IL_6RA	1.16	0.82, 1.65	0.4	1.16	0.97, 1.38	0.1	0.81	0.72, 0.90	<0.001	1.36	0.80, 2.32	0.3	<0.001	<0.001
CHIT1	1.05	0.89, 1.23	0.6	1.03	0.95, 1.11	0.5	0.89	0.84, 0.93	<0.001	1.02	0.81, 1.28	0.9	<0.001	<0.001
EP_CAM	1.08	0.87, 1.33	0.5	0.99	0.89, 1.11	0.9	0.78	0.72, 0.85	<0.001	0.97	0.71, 1.34	0.9	<0.001	<0.001
PCSK9	1.15	0.78, 1.68	0.5	1.02	0.84, 1.24	0.8	0.73	0.63, 0.83	<0.001	1.24	0.71, 2.19	0.5	<0.001	<0.001
EGFR	1.28	0.84, 1.95	0.3	0.88	0.70, 1.09	0.2	0.64	0.55, 0.75	<0.001	0.78	0.41, 1.50	0.5	<0.001	<0.001
RARRES2	1.04	0.69, 1.54	0.9	1.15	0.93, 1.41	0.2	0.76	0.67, 0.86	<0.001	1.25	0.66, 2.38	0.5	<0.001	<0.001
SRC	0.98	0.82, 1.17	0.8	0.95	0.87, 1.04	0.2	0.9	0.84, 0.96	<0.001	0.86	0.66, 1.13	0.3	0.018	0.024
IDUA	0.98	0.64, 1.51	0.9	0.81	0.66, 1.00	0.055	0.8	0.69, 0.93	0.003	0.65	0.35, 1.18	0.2	0.016	0.021
LOX_1	0.73	0.52, 1.03	0.074	0.93	0.79, 1.10	0.4	1.39	1.24, 1.55	<0.001	0.94	0.57, 1.54	0.8	<0.001	<0.001
PIgR	2.39	0.43, 13.2	0.3	1.15	0.50, 2.64	0.7	3.71	2.02, 6.80	<0.001	11.4	0.90, 146	0.061	<0.001	<0.001
SERPINA12	0.83	0.66, 1.04	0.1	1.04	0.93, 1.15	0.5	1.14	1.06, 1.23	<0.001	1.14	0.84, 1.55	0.4	0.002	0.003
AMBP	2.01	0.96, 4.23	0.065	0.98	0.68, 1.40	0.9	0.48	0.37, 0.61	<0.001	1.97	0.64, 6.00	0.2	<0.001	<0.001
IL16	1.26	0.89, 1.79	0.2	1.17	0.98, 1.39	0.08	1.25	1.10, 1.41	<0.001	1.61	0.98, 2.67	0.062	0.002	0.003
IgG Fc receptor IIb	1.13	0.91, 1.42	0.3	1.12	1.00, 1.25	0.053	1.1	1.02, 1.19	0.018	1.4	0.98, 2.00	0.064	0.019	0.025
GDF_2	1	0.74, 1.36	0.9	0.95	0.81, 1.10	0.5	0.84	0.76, 0.94	0.002	1.04	0.66, 1.64	0.9	0.037	0.047
MARCO	1.29	0.53, 3.15	0.6	1.02	0.65, 1.59	0.9	0.44	0.33, 0.60	<0.001	1.02	0.27, 3.90	0.9	<0.001	<0.001
MMP_12	0.96	0.75, 1.22	0.7	0.93	0.82, 1.05	0.2	0.85	0.78, 0.92	<0.001	1.21	0.84, 1.74	0.3	0.003	0.004
TGM2	0.82	0.61, 1.10	0.2	0.91	0.78, 1.06	0.2	1.37	1.22, 1.54	<0.001	1.02	0.64, 1.62	0.9	<0.001	<0.001
CASA	0.89	0.71, 1.12	0.3	1.1	0.99, 1.21	0.064	1.23	1.15, 1.31	<0.001	1.25	0.96, 1.63	0.1	<0.001	<0.001
PARP_1	0.77	0.56, 1.06	0.11	0.96	0.84, 1.11	0.6	1.18	1.09, 1.29	<0.001	0.95	0.62, 1.46	0.8	<0.001	<0.001
HAOX1	0.87	0.75, 1.00	0.058	0.99	0.93, 1.06	0.9	1.11	1.06, 1.16	<0.001	0.9	0.73, 1.11	0.3	<0.001	<0.001
ZBTB16	0.77	0.58, 1.03	0.083	0.9	0.79, 1.03	0.11	1.16	1.06, 1.25	<0.001	0.89	0.59, 1.33	0.6	<0.001	<0.001
TPSAB1	1.45	0.99, 2.12	0.057	1.04	0.86, 1.25	0.7	0.87	0.76, 0.99	0.036	0.64	0.37, 1.10	0.11	0.021	0.027
IRAK1	0.77	0.52, 1.14	0.2	0.88	0.73, 1.06	0.2	1.21	1.08, 1.36	<0.001	1.16	0.72, 1.88	0.5	0.002	0.003
TRIM5	0.69	0.44, 1.10	0.12	0.94	0.76, 1.15	0.6	1.36	1.20, 1.55	<0.001	1.05	0.58, 1.90	0.9	<0.001	<0.001
DCTN1	0.85	0.67, 1.07	0.2	0.9	0.80, 1.01	0.075	1.13	1.04, 1.22	0.002	0.87	0.61, 1.23	0.4	<0.001	0.002
TRAF2	0.67	0.44, 1.03	0.07	0.98	0.81, 1.18	0.8	1.19	1.05, 1.35	0.006	0.78	0.42, 1.43	0.4	0.01	0.014
TRIM21	0.84	0.62, 1.14	0.3	0.86	0.74, 1.00	0.053	1.17	1.06, 1.29	<0.001	0.78	0.49, 1.24	0.3	<0.001	<0.001
EGLN1	0.9	0.52, 1.54	0.7	1.1	0.86, 1.41	0.4	1.88	1.62, 2.18	<0.001	1.17	0.58, 2.38	0.7	<0.001	<0.001
NFATC3	0.93	0.66, 1.30	0.7	0.95	0.80, 1.12	0.5	1.26	1.14, 1.38	<0.001	1.02	0.64, 1.62	0.9	<0.001	<0.001
EIF4G1	0.86	0.71, 1.03	0.1	0.94	0.86, 1.03	0.2	1.09	1.03, 1.16	0.005	1.01	0.77, 1.32	0.9	0.006	0.008
HSD11B1	0.68	0.42, 1.09	0.11	0.96	0.76, 1.22	0.7	1.79	1.51, 2.11	<0.001	1.17	0.57, 2.37	0.7	<0.001	<0.001
HEXIM1	0.85	0.67, 1.08	0.2	0.96	0.86, 1.07	0.5	1.14	1.05, 1.23	<0.001	1.04	0.75, 1.45	0.8	0.004	0.006
BACH1	1.01	0.66, 1.54	0.9	1.01	0.82, 1.25	0.9	1.27	1.11, 1.46	<0.001	0.89	0.46, 1.73	0.7	0.015	0.021
PIK3AP1	0.8	0.60, 1.07	0.13	0.96	0.84, 1.10	0.6	1.16	1.06, 1.27	<0.001	0.88	0.58, 1.34	0.6	0.004	0.006
DFFA	0.78	0.57, 1.05	0.1	0.96	0.84, 1.10	0.5	1.22	1.12, 1.34	<0.001	1.01	0.68, 1.51	0.9	<0.001	<0.001
MASP1	0.94	0.49, 1.79	0.9	1.26	0.91, 1.75	0.2	1.77	1.41, 2.23	<0.001	0.78	0.30, 2.05	0.6	<0.001	<0.001
CEACAM1	0.42	0.17, 1.03	0.057	1.33	0.76, 2.33	0.3	3.76	2.45, 5.75	<0.001	1.79	0.32, 10.0	0.5	<0.001	<0.001
GPC1	1.31	0.81, 2.12	0.3	1	0.79, 1.28	0.9	1.49	1.25, 1.76	<0.001	1.68	0.82, 3.41	0.2	<0.001	<0.001
ERBB2	0.76	0.44, 1.29	0.3	1.02	0.78, 1.35	0.9	1.42	1.16, 1.73	<0.001	0.95	0.42, 2.16	0.9	0.006	0.009
MetAP 2	0.85	0.62, 1.15	0.3	0.96	0.82, 1.12	0.6	1.18	1.07, 1.32	0.002	0.97	0.61, 1.53	0.9	0.011	0.015
FASLG	1.38	0.92, 2.06	0.12	1.15	0.94, 1.41	0.2	0.86	0.75, 0.98	0.029	0.86	0.48, 1.54	0.6	0.024	0.031
Gal-1	1.71	0.80, 3.66	0.2	1.2	0.79, 1.81	0.4	0.69	0.52, 0.92	0.012	1.1	0.32, 3.80	0.9	0.035	0.045
ERBB4	0.73	0.41, 1.32	0.3	1.01	0.74, 1.36	0.9	1.82	1.46, 2.27	<0.001	1.06	0.42, 2.63	0.9	<0.001	<0.001
S100A11	0.78	0.39, 1.54	0.5	1.26	0.93, 1.70	0.14	1.71	1.40, 2.10	<0.001	2.03	0.99, 4.15	0.054	<0.001	<0.001
ICOSLG	1.25	0.68, 2.29	0.5	0.82	0.60, 1.11	0.2	0.72	0.57, 0.89	0.003	1.47	0.60, 3.56	0.4	0.02	0.026
ANXA1	0.81	0.55, 1.20	0.3	1.03	0.86, 1.22	0.8	1.28	1.14, 1.43	<0.001	1.17	0.72, 1.88	0.5	<0.001	<0.001
MPO	1.22	0.88, 1.69	0.2	1.21	1.03, 1.43	0.022	0.92	0.82, 1.03	0.2	1.2	0.73, 1.96	0.5	0.035	0.045
ITGA6	0.83	0.61, 1.13	0.2	0.71	0.60, 0.84	<0.001	1.03	0.94, 1.13	0.5	0.81	0.51, 1.30	0.4	<0.001	<0.001
NF2	0.85	0.69, 1.04	0.11	0.87	0.79, 0.96	0.007	1.02	0.95, 1.08	0.6	0.79	0.57, 1.09	0.2	0.009	0.013
SPRY2	0.82	0.66, 1.01	0.065	0.89	0.81, 0.99	0.029	1.04	0.97, 1.10	0.3	0.88	0.65, 1.20	0.4	0.022	0.029
ICA1	0.65	0.40, 1.06	0.084	0.76	0.60, 0.94	0.013	1.04	0.91, 1.18	0.6	0.76	0.38, 1.48	0.4	0.021	0.027
S100A4	0.85	0.56, 1.29	0.4	0.69	0.56, 0.85	<0.001	1	0.86, 1.15	0.9	0.66	0.35, 1.24	0.2	0.006	0.008
FURIN	0.97	0.61, 1.54	0.9	1.28	1.01, 1.62	0.039	0.89	0.75, 1.05	0.2	0.57	0.29, 1.11	0.1	0.037	0.046
REN	0.74	0.60, 0.91	0.004	1	0.89, 1.12	0.9	1.04	0.96, 1.13	0.3	0.8	0.58, 1.09	0.2	0.025	0.032
DECR1	0.8	0.66, 0.97	0.02	0.92	0.85, 1.01	0.082	1.06	1.00, 1.12	0.064	1.03	0.80, 1.33	0.8	0.007	0.01
DDX58	0.67	0.46, 0.97	0.035	0.92	0.78, 1.07	0.3	1.1	1.00, 1.22	0.055	0.68	0.39, 1.18	0.2	0.01	0.013
gal_4	1.2	0.92, 1.56	0.2	1.13	0.98, 1.29	0.083	1.01	0.92, 1.10	0.9	1.65	1.12, 2.41	0.011	0.032	0.041
pon3	1.26	1.00, 1.58	0.051	0.86	0.77, 0.95	0.005	0.64	0.59, 0.69	<0.001	0.85	0.62, 1.18	0.3	<0.001	<0.001
CD40_ligand	0.87	0.72, 1.05	0.2	0.9	0.82, 0.98	0.022	0.92	0.87, 0.99	0.019	0.84	0.62, 1.12	0.2	0.018	0.024
IL-1ra	1.06	0.82, 1.38	0.6	1.42	1.25, 1.61	<0.001	1.22	1.12, 1.34	<0.001	1.1	0.74, 1.62	0.6	<0.001	<0.001
TIE2	0.86	0.45, 1.63	0.6	1.6	1.14, 2.26	0.007	2.62	2.04, 3.37	<0.001	1.92	0.67, 5.46	0.2	<0.001	<0.001
IL1RL2	1.12	0.71, 1.77	0.6	1.46	1.16, 1.83	<0.001	0.8	0.68, 0.94	0.006	0.83	0.42, 1.64	0.6	<0.001	<0.001
GIF	0.98	0.82, 1.17	0.8	0.9	0.82, 0.98	0.013	0.87	0.82, 0.92	<0.001	0.92	0.71, 1.19	0.5	<0.001	<0.001
CNTNAP2	1.11	0.72, 1.70	0.6	1.34	1.09, 1.64	0.005	1.19	1.02, 1.38	0.026	1.41	0.79, 2.51	0.2	0.023	0.03

Biomarker	Pulm Tissue congestion with HFpEF			Pulm tissue and Syst tissue congestion with AF			Pulm IV congestion with dilated LA & LV			Global congestion			P overall	P-corrected
(Olink name)	OR	CI	p	OR	CI	p	OR	CI	p	OR	CI	p		
EDAR	0.9	0.62, 1.29	0.6	0.69	0.56, 0.84	<0.001	0.78	0.69, 0.90	<0.001	0.73	0.41, 1.33	0.3	<0.001	<0.001
GALNT3	0.77	0.48, 1.23	0.3	1.33	1.07, 1.65	0.009	1.65	1.42, 1.91	<0.001	1.69	0.93, 3.07	0.084	<0.001	<0.001
HNMT	1.05	0.77, 1.42	0.8	1.3	1.12, 1.51	<0.001	1.47	1.33, 1.63	<0.001	1.45	0.96, 2.22	0.081	<0.001	<0.001
KLRD1	1.15	0.83, 1.58	0.4	1.33	1.13, 1.57	<0.001	1.14	1.01, 1.27	0.028	1.56	0.97, 2.52	0.069	0.002	0.002
STC1	0.93	0.59, 1.45	0.7	1.94	1.48, 2.53	<0.001	1.76	1.46, 2.12	<0.001	1.23	0.58, 2.61	0.6	<0.001	<0.001
KLK13	0.77	0.50, 1.19	0.2	0.68	0.55, 0.85	<0.001	0.84	0.73, 0.98	0.027	1.18	0.64, 2.18	0.6	0.003	0.005
EGF	0.91	0.78, 1.05	0.2	0.92	0.85, 0.99	0.018	0.94	0.90, 0.99	0.024	0.82	0.66, 1.03	0.088	0.013	0.017
MIA	0.67	0.36, 1.25	0.2	0.63	0.46, 0.86	0.003	0.71	0.56, 0.89	0.003	0.58	0.23, 1.42	0.2	0.003	0.004
CD207	1.3	0.84, 2.00	0.2	0.79	0.63, 0.99	0.038	0.79	0.67, 0.92	0.002	0.85	0.44, 1.65	0.6	0.007	0.01
ADAM-TS 15	1.24	0.86, 1.78	0.3	1.49	1.23, 1.79	<0.001	1.15	1.01, 1.31	0.028	1.09	0.64, 1.86	0.8	<0.001	<0.001
CEACAM5	1.1	0.84, 1.44	0.5	1.28	1.12, 1.45	<0.001	1.22	1.11, 1.34	<0.001	1.36	0.95, 1.93	0.091	<0.001	<0.001
PAI	0.69	0.57, 0.84	<0.001	1.01	0.92, 1.11	0.8	1.12	1.05, 1.19	<0.001	0.79	0.59, 1.05	0.1	<0.001	<0.001
TR_AP	1.46	1.08, 1.96	0.012	0.94	0.81, 1.10	0.5	0.53	0.47, 0.59	<0.001	0.97	0.61, 1.53	0.9	<0.001	<0.001
CCL22	1.22	1.03, 1.45	0.024	1.09	0.99, 1.20	0.08	0.78	0.72, 0.85	<0.001	0.91	0.65, 1.27	0.6	<0.001	<0.001
SORT1	0.53	0.32, 0.85	0.009	1.34	0.97, 1.86	0.072	1.59	1.27, 2.01	<0.001	1	0.39, 2.56	0.9	<0.001	<0.001
PSIP1	0.71	0.50, 0.99	0.041	1.01	0.88, 1.15	0.9	1.34	1.24, 1.45	<0.001	1.2	0.86, 1.68	0.3	<0.001	<0.001
CLEC4A	1.88	1.15, 3.07	0.012	0.96	0.75, 1.23	0.7	0.7	0.59, 0.84	<0.001	0.74	0.35, 1.56	0.4	<0.001	<0.001
PRDX3	0.7	0.49, 0.99	0.046	0.91	0.78, 1.07	0.2	1.16	1.05, 1.28	0.004	0.9	0.56, 1.45	0.7	0.002	0.003
PADI2	0.41	0.21, 0.79	0.007	0.9	0.72, 1.12	0.4	1.26	1.11, 1.43	<0.001	1.34	0.84, 2.14	0.2	<0.001	<0.001
ITGB6	0.58	0.35, 0.96	0.033	0.82	0.64, 1.06	0.13	1.53	1.28, 1.83	<0.001	1.98	0.96, 4.06	0.063	<0.001	<0.001
CPE	0.51	0.32, 0.81	0.004	1	0.79, 1.28	0.9	1.73	1.45, 2.06	<0.001	1.53	0.75, 3.15	0.2	<0.001	<0.001
LDL_RECEPTOR	1.25	0.97, 1.60	0.079	0.95	0.83, 1.08	0.4	0.61	0.56, 0.67	<0.001	0.68	0.46, 1.00	0.049	<0.001	<0.001
MEPE	1.32	1.00, 1.75	0.052	0.89	0.77, 1.03	0.12	0.82	0.74, 0.91	<0.001	1.6	1.06, 2.42	0.024	<0.001	<0.001
CPA1	0.99	0.81, 1.21	0.9	1.05	0.95, 1.16	0.3	1.09	1.02, 1.17	0.013	1.39	1.04, 1.85	0.027	0.03	0.038
JAM_A	0.83	0.66, 1.04	0.1	0.98	0.88, 1.09	0.7	1.1	1.03, 1.19	0.007	1.34	1.02, 1.75	0.034	0.004	0.006
SLAMF7	0.82	0.61, 1.10	0.2	1.05	0.91, 1.22	0.5	1.43	1.29, 1.58	<0.001	1.84	1.27, 2.67	<0.001	<0.001	<0.001
Brother of CDO	0.72	0.40, 1.30	0.3	1.07	0.80, 1.43	0.7	3.46	2.80, 4.26	<0.001	4.14	1.83, 9.39	<0.001	<0.001	<0.001
RAGE	0.97	0.65, 1.43	0.9	1.01	0.83, 1.23	0.9	1.99	1.71, 2.32	<0.001	3.86	1.89, 7.86	<0.001	<0.001	<0.001
CD84	0.88	0.55, 1.41	0.6	1.21	0.96, 1.53	0.1	1.46	1.24, 1.72	<0.001	2.09	1.06, 4.11	0.034	<0.001	<0.001
VSIG2	1.11	0.83, 1.49	0.5	1.11	0.96, 1.28	0.2	1.31	1.18, 1.45	<0.001	2.01	1.34, 3.01	<0.001	<0.001	<0.001
CEACAM8	0.87	0.64, 1.18	0.4	1.15	1.00, 1.33	0.055	1.61	1.46, 1.78	<0.001	1.86	1.26, 2.73	0.002	<0.001	<0.001
Dkk1	0.77	0.58, 1.03	0.081	1.03	0.89, 1.19	0.7	1.33	1.20, 1.47	<0.001	2.08	1.39, 3.10	<0.001	<0.001	<0.001
GT	1.08	0.81, 1.45	0.6	1.07	0.92, 1.23	0.4	1.12	1.01, 1.25	0.025	1.81	1.34, 2.45	<0.001	0.004	0.006
ACE2	0.73	0.53, 1.00	0.052	1.12	0.96, 1.31	0.14	1.73	1.55, 1.92	<0.001	1.96	1.29, 2.98	0.002	<0.001	<0.001
TNFRSF13B	0.78	0.53, 1.16	0.2	1.04	0.86, 1.25	0.7	1.37	1.20, 1.56	<0.001	2.38	1.62, 3.51	<0.001	<0.001	<0.001
HSP 27	1.12	0.71, 1.78	0.6	1.15	0.91, 1.46	0.2	1.29	1.09, 1.52	0.003	2.61	1.08, 6.30	0.033	0.006	0.009
VEGFD	0.79	0.53, 1.17	0.2	0.91	0.73, 1.13	0.4	2.6	2.14, 3.17	<0.001	3.19	1.34, 7.56	0.008	<0.001	<0.001
CLEC4C	1.15	0.82, 1.62	0.4	0.93	0.78, 1.10	0.4	0.69	0.61, 0.77	<0.001	0.52	0.32, 0.85	0.009	<0.001	<0.001
CXCL12	0.83	0.31, 2.20	0.7	1.22	0.76, 1.98	0.4	2.33	1.67, 3.26	<0.001	4.42	1.31, 14.9	0.016	<0.001	<0.001
LAMP3	0.89	0.64, 1.23	0.5	1.1	0.94, 1.29	0.3	1.15	1.03, 1.29	0.015	2.21	1.36, 3.59	<0.001	0.002	0.003
MSLN	1.09	0.82, 1.45	0.5	1.15	1.0, 1.32	0.059	1.35	1.23, 1.49	<0.001	2.31	1.56, 3.43	<0.001	<0.001	<0.001
IGF1R	0.86	0.50, 1.49	0.6	1.3	1.00, 1.69	0.05	3.04	2.53, 3.64	<0.001	6.23	3.60, 10.8	<0.001	<0.001	<0.001
CYR61	1.07	0.77, 1.50	0.7	0.98	0.83, 1.16	0.8	1.48	1.32, 1.67	<0.001	1.78	1.10, 2.88	0.02	<0.001	<0.001
PVRL4	1.19	0.85, 1.65	0.3	1.13	0.96, 1.34	0.14	1.27	1.13, 1.43	<0.001	2.71	1.76, 4.16	<0.001	<0.001	<0.001
ABL1	0.86	0.65, 1.14	0.3	1.05	0.92, 1.19	0.5	1.25	1.15, 1.36	<0.001	1.44	1.05, 1.97	0.022	<0.001	<0.001
RET	0.9	0.64, 1.28	0.6	0.95	0.80, 1.14	0.6	0.52	0.46, 0.59	<0.001	0.41	0.24, 0.68	<0.001	<0.001	<0.001
CRNN	1.12	0.89, 1.39	0.3	1.11	0.99, 1.24	0.075	0.87	0.80, 0.94	<0.001	1.4	1.04, 1.87	0.025	<0.001	<0.001
CD160	1.29	0.95, 1.74	0.1	1.06	0.91, 1.24	0.5	1.12	1.01, 1.25	0.04	1.61	1.04, 2.49	0.033	0.04	0.05
TNFRSF4	1.06	0.77, 1.47	0.7	1.12	0.95, 1.32	0.2	1.21	1.08, 1.36	<0.001	2.2	1.44, 3.38	<0.001	<0.001	<0.001
MAD homolog 5	1.13	0.48, 2.65	0.8	0.61	0.32, 1.16	0.13	0.3	0.19, 0.47	<0.001	0.19	0.04, 0.90	0.036	<0.001	<0.001
FR-alpha	1.22	0.86, 1.73	0.3	1.16	0.98, 1.39	0.09	1.31	1.16, 1.48	<0.001	2.88	1.79, 4.63	<0.001	<0.001	<0.001
casp_3	0.8	0.68, 0.94	0.008	0.89	0.83, 0.97	0.005	0.99	0.94, 1.05	0.8	0.82	0.64, 1.05	0.11	0.002	0.003
MMP_7	1.47	1.02, 2.11	0.039	1.22	1.03, 1.45	0.022	0.95	0.85, 1.05	0.3	1.51	0.87, 2.63	0.14	0.005	0.007
HB_EGF	0.59	0.36, 0.95	0.029	0.73	0.59, 0.91	0.005	0.99	0.86, 1.14	0.9	0.6	0.29, 1.22	0.2	0.004	0.006
GLB1	0.56	0.35, 0.91	0.019	0.77	0.61, 0.96	0.019	1.07	0.94, 1.23	0.3	0.76	0.39, 1.48	0.4	0.006	0.008
FADD	0.74	0.56, 0.99	0.041	0.86	0.75, 0.98	0.021	1.06	0.98, 1.15	0.2	0.93	0.64, 1.36	0.7	0.008	0.011
RETN	1.11	0.84, 1.46	0.5	1.23	1.08, 1.41	0.003	1.07	0.98, 1.18	0.14	2.32	1.59, 3.38	<0.001	<0.001	<0.001
PSP_D	0.97	0.75, 1.25	0.8	0.85	0.74, 0.97	0.015	0.97	0.88, 1.06	0.5	1.51	1.07, 2.15	0.021	0.019	0.025
FAS	1.33	0.97, 1.81	0.074	1.23	1.05, 1.44	0.009	0.96	0.86, 1.08	0.5	2.19	1.47, 3.25	<0.001	<0.001	<0.001
PRTN3	1.3	0.99, 1.69	0.056	1.21	1.06, 1.39	0.006	1.08	0.97, 1.19	0.15	1.56	1.09, 2.23	0.016	0.005	0.007
SHPS_1	1.32	0.98, 1.78	0.072	1.31	1.13, 1.52	<0.001	1.1	0.99, 1.23	0.065	2.58	1.72, 3.85	<0.001	<0.001	<0.001
SCGB3A2	1.09	0.91, 1.30	0.4	1.22	1.13, 1.33	<0.001	1.06	0.99, 1.13	0.093	1.46	1.20, 1.77	<0.001	<0.001	<0.001
CD93	1.25	0.89, 1.77	0.2	1.19	1.00, 1.41	0.044	1.11	0.99, 1.24	0.083	3.27	1.82, 5.87	<0.001	<0.001	<0.001
ICAM_2	1.29	0.95, 1.75	0.1	1.31	1.13, 1.53	<0.001	1.09	0.98, 1.21	0.11	2.46	1.60, 3.79	<0.001	<0.001	<0.001
LPL	1.13	0.73, 1.75	0.6	1.8	1.43, 2.28	<0.001	1.12	0.96, 1.30	0.2	2.8	1.33, 5.89	0.007	<0.001	<0.001
PRKCQ	1.34	0.94, 1.91	0.11	0.78	0.62, 0.98	0.033	0.95	0.82, 1.10	0.5	0.39	0.17, 0.87	0.022	0.01	0.014
SIT1	0.93	0.63, 1.38	0.7	0.79	0.64, 0.97	0.021	0.97	0.85, 1.12	0.7	0.28	0.15, 0.54	<0.001	<0.001	<0.001
CTSV	0.77	0.52, 1.14	0.2	0.7	0.57, 0.85	<0.001	0.89	0.78, 1.02	0.091	0.35	0.19, 0.63	<0.001	<0.001	<0.001
CDH5	1.38	1.02, 1.88	0.036	1.14	0.97, 1.33	0.1	0.95	0.85, 1.06	0.4	1.96	1.28, 3.02	0.002	0.002	0.003
SELE	1.58	1.21, 2.07	<0.001	1.28	1.12, 1.46	<0.001	0.89	0.81, 0.98	0.019	1.48	0.99, 2.22	0.055	<0.001	<0.001
SCF	1.7	1.12, 2.58	0.013	0.83	0.70, 0.99	0.033	0.5	0.44, 0.56	<0.001	0.65	0.40, 1.05	0.078	<0.001	<0.001
GLO1	0.74	0.54, 1.00	0.048	0.77	0.67, 0.90	<0.001	1.16	1.04, 1.28	0.006	0.77	0.49, 1.22	0.3	<0.001	<0.001

Biomarker	Pulm Tissue congestion with HFpEF			Pulm tissue and Syst tissue congestion with AF			Pulm IV congestion with dilated LA & LV			Global congestion			P overall	P-corrected
(Olink name)	OR	CI	p	OR	CI	p	OR	CI	p	OR	CI	p		
PRDX1	0.69	0.48, 1.00	0.047	0.79	0.67, 0.93	0.005	1.16	1.06, 1.27	<0.001	0.86	0.53, 1.38	0.5	<0.001	<0.001
SCF	1.67	1.12, 2.50	0.012	0.83	0.70, 0.98	0.028	0.5	0.44, 0.56	<0.001	0.69	0.43, 1.12	0.14	<0.001	<0.001
FGF-BP1	0.68	0.48, 0.96	0.028	1.14	1.03, 1.26	0.009	1.35	1.27, 1.44	<0.001	1.25	0.96, 1.63	0.095	<0.001	<0.001
OPG	1.31	0.96, 1.80	0.089	1.45	1.24, 1.69	<0.001	1.2	1.07, 1.33	<0.001	2.76	1.83, 4.15	<0.001	<0.001	<0.001
CD163	1.3	0.98, 1.74	0.07	1.45	1.25, 1.67	<0.001	1.15	1.05, 1.27	0.004	2.51	1.61, 3.91	<0.001	<0.001	<0.001
GRN	1.33	0.92, 1.92	0.13	1.41	1.17, 1.70	<0.001	1.22	1.07, 1.38	0.002	2.84	1.66, 4.88	<0.001	<0.001	<0.001
PLC	1.32	0.97, 1.79	0.078	1.52	1.30, 1.78	<0.001	1.33	1.19, 1.48	<0.001	4.05	2.53, 6.49	<0.001	<0.001	<0.001
TIMP4	1.29	0.97, 1.71	0.077	1.48	1.28, 1.70	<0.001	1.15	1.05, 1.27	0.004	2.6	1.82, 3.71	<0.001	<0.001	<0.001
TR	1.08	0.82, 1.42	0.6	1.63	1.42, 1.87	<0.001	1.62	1.47, 1.79	<0.001	2.45	1.65, 3.61	<0.001	<0.001	<0.001
TNFRSF10C	1.32	0.98, 1.77	0.067	1.22	1.05, 1.41	0.008	0.9	0.82, 0.99	0.032	1.71	1.09, 2.67	0.019	<0.001	<0.001
GDF_15	1.06	0.85, 1.32	0.6	1.47	1.32, 1.64	<0.001	1.6	1.48, 1.74	<0.001	2.52	1.92, 3.31	<0.001	<0.001	<0.001
SPON1	1.33	0.95, 1.86	0.1	1.57	1.34, 1.85	<0.001	1.61	1.43, 1.81	<0.001	3.29	2.36, 4.58	<0.001	<0.001	<0.001
CXCL16	1.26	0.91, 1.75	0.2	1.47	1.24, 1.73	<0.001	1.12	1.00, 1.26	0.044	2.51	1.57, 4.00	<0.001	<0.001	<0.001
IGFBP	0.88	0.75, 1.04	0.13	1.12	1.03, 1.21	0.008	1.49	1.40, 1.58	<0.001	2.03	1.57, 2.63	<0.001	<0.001	<0.001
AP_N	1.2	0.87, 1.66	0.3	1.22	1.04, 1.43	0.017	1.46	1.30, 1.64	<0.001	2.68	1.67, 4.28	<0.001	<0.001	<0.001
AXL	1.26	0.91, 1.72	0.2	1.38	1.17, 1.61	<0.001	1.17	1.04, 1.30	0.006	1.91	1.20, 3.05	0.006	<0.001	<0.001
MMP_2	1.1	0.83, 1.46	0.5	1.18	1.02, 1.36	0.022	1.34	1.22, 1.49	<0.001	2.49	1.67, 3.73	<0.001	<0.001	<0.001
TNFSF13B	1.31	1.00, 1.72	0.051	1.46	1.27, 1.67	<0.001	1.16	1.06, 1.28	0.002	1.98	1.36, 2.88	<0.001	<0.001	<0.001
U_PAR	1.18	0.88, 1.59	0.3	1.62	1.40, 1.88	<0.001	1.48	1.34, 1.65	<0.001	3.99	2.67, 5.95	<0.001	<0.001	<0.001
OPN	0.97	0.76, 1.22	0.8	1.37	1.21, 1.55	<0.001	1.51	1.39, 1.66	<0.001	2.91	1.98, 4.27	<0.001	<0.001	<0.001
CTSD	1.15	0.83, 1.60	0.4	1.42	1.21, 1.67	<0.001	1.34	1.20, 1.50	<0.001	2.13	1.34, 3.38	<0.001	<0.001	<0.001
PGLYRP1	1.23	0.94, 1.61	0.14	1.2	1.05, 1.37	0.009	1.11	1.01, 1.22	0.034	1.87	1.27, 2.77	0.002	<0.001	<0.001
CCL15	1.07	0.81, 1.42	0.6	1.34	1.17, 1.55	<0.001	1.27	1.15, 1.40	<0.001	2.37	1.62, 3.49	<0.001	<0.001	<0.001
ST2	1.06	0.84, 1.34	0.6	1.58	1.41, 1.76	<0.001	1.82	1.68, 1.97	<0.001	3.14	2.37, 4.15	<0.001	<0.001	<0.001
IGFBP	1.14	0.88, 1.47	0.3	1.38	1.22, 1.57	<0.001	1.58	1.44, 1.73	<0.001	2.46	1.79, 3.39	<0.001	<0.001	<0.001
IGFBP_2	1.12	0.87, 1.42	0.4	1.39	1.23, 1.58	<0.001	1.53	1.39, 1.67	<0.001	4.33	2.65, 7.06	<0.001	<0.001	<0.001
BMP_6	0.87	0.68, 1.11	0.3	1.38	1.19, 1.59	<0.001	1.72	1.55, 1.91	<0.001	2.36	1.53, 3.64	<0.001	<0.001	<0.001
ADM	1.14	0.85, 1.54	0.4	2.11	1.75, 2.55	<0.001	2.42	2.11, 2.78	<0.001	7.26	3.71, 14.2	<0.001	<0.001	<0.001
Placenta growth factor	1.02	0.69, 1.50	0.9	1.29	1.06, 1.57	0.009	1.34	1.17, 1.54	<0.001	2.81	1.67, 4.72	<0.001	<0.001	<0.001
ADAM-TS13	0.53	0.20, 1.40	0.2	0.28	0.18, 0.46	<0.001	0.21	0.15, 0.30	<0.001	0.05	0.01, 0.14	<0.001	<0.001	<0.001
IL-4RA	0.96	0.63, 1.46	0.9	1.59	1.31, 1.92	<0.001	2.3	2.01, 2.63	<0.001	4.19	2.76, 6.35	<0.001	<0.001	<0.001
IL6	0.91	0.74, 1.11	0.3	1.33	1.21, 1.45	<0.001	1.55	1.46, 1.65	<0.001	1.85	1.50, 2.29	<0.001	<0.001	<0.001
TNFRSF10A	1.11	0.73, 1.69	0.6	1.79	1.45, 2.20	<0.001	2.1	1.81, 2.43	<0.001	4.75	2.74, 8.25	<0.001	<0.001	<0.001
TNFRSF11A	1.13	0.85, 1.50	0.4	1.51	1.31, 1.74	<0.001	1.59	1.44, 1.76	<0.001	2.81	1.93, 4.09	<0.001	<0.001	<0.001
PAR_1	1.19	0.78, 1.82	0.4	1.36	1.10, 1.68	0.004	1.75	1.50, 2.04	<0.001	4.41	2.16, 9.00	<0.001	<0.001	<0.001
TRAIL_R2	0.96	0.68, 1.35	0.8	1.53	1.30, 1.80	<0.001	2.23	1.99, 2.50	<0.001	3.23	2.42, 4.32	<0.001	<0.001	<0.001
TF	1.35	0.80, 2.27	0.3	1.57	1.20, 2.03	<0.001	1.66	1.38, 2.00	<0.001	5.57	2.60, 11.9	<0.001	<0.001	<0.001
PDGF subunit B	0.9	0.78, 1.05	0.2	0.93	0.86, 1.00	0.048	0.95	0.90, 1.00	0.04	0.79	0.63, 0.99	0.04	0.02	0.026
IL_27	0.97	0.62, 1.52	0.9	2.01	1.61, 2.52	<0.001	3.08	2.61, 3.63	<0.001	6.23	3.23, 12.0	<0.001	<0.001	<0.001
IL_17D	0.89	0.50, 1.58	0.7	1.42	1.10, 1.82	0.006	1.29	1.07, 1.55	0.008	2.05	1.25, 3.34	0.004	0.003	0.004
GAL_9	1.19	0.66, 2.16	0.6	2.23	1.65, 3.02	<0.001	2.33	1.88, 2.88	<0.001	5.52	2.22, 13.7	<0.001	<0.001	<0.001
FGF_21	0.94	0.82, 1.09	0.4	1.22	1.14, 1.30	<0.001	1.33	1.27, 1.40	<0.001	1.46	1.22, 1.75	<0.001	<0.001	<0.001
FGF_23	0.9	0.76, 1.07	0.2	1.36	1.28, 1.46	<0.001	1.59	1.51, 1.66	<0.001	1.91	1.61, 2.25	<0.001	<0.001	<0.001
SPON2	1.12	0.39, 3.19	0.8	4.92	2.71, 8.93	<0.001	14	8.93, 22.0	<0.001	427	47.5, 3,841	<0.001	<0.001	<0.001
GH	0.92	0.81, 1.04	0.2	1.1	1.02, 1.17	0.007	1.31	1.25, 1.38	<0.001	1.55	1.22, 1.96	<0.001	<0.001	<0.001
FS	0.8	0.55, 1.17	0.3	1.34	1.11, 1.63	0.003	2.26	1.96, 2.60	<0.001	2.49	1.41, 4.39	0.002	<0.001	<0.001
PAPPA	0.76	0.54, 1.05	0.1	1.24	1.06, 1.45	0.006	1.75	1.58, 1.95	<0.001	2.23	1.53, 3.25	<0.001	<0.001	<0.001
MERTK	0.99	0.65, 1.50	0.9	1.24	1.01, 1.54	0.043	1.43	1.23, 1.66	<0.001	2.29	1.21, 4.32	0.011	<0.001	<0.001
KIM1	1.19	0.95, 1.48	0.13	1.24	1.11, 1.38	<0.001	1.39	1.28, 1.50	<0.001	1.45	1.06, 1.99	0.02	<0.001	<0.001
THBS2	0.62	0.27, 1.41	0.3	3.31	2.15, 5.09	<0.001	3.28	2.42, 4.46	<0.001	11.6	3.15, 42.9	<0.001	<0.001	<0.001
Prolargin	1.15	0.55, 2.41	0.7	4.13	2.82, 6.04	<0.001	9.15	6.90, 12.1	<0.001	19.3	6.62, 56.5	<0.001	<0.001	<0.001
Heme oxygenase 1	0.94	0.61, 1.44	0.8	1.32	1.06, 1.66	0.015	1.92	1.63, 2.26	<0.001	3.69	1.79, 7.64	<0.001	<0.001	<0.001
Lymphotactin	0.96	0.68, 1.36	0.8	1.47	1.24, 1.75	<0.001	1.52	1.35, 1.72	<0.001	2.88	1.86, 4.48	<0.001	<0.001	<0.001
PTX3	0.79	0.56, 1.13	0.2	1.65	1.38, 1.98	<0.001	2.56	2.24, 2.92	<0.001	3.69	2.19, 6.19	<0.001	<0.001	<0.001
PSGL_1	0.81	0.37, 1.77	0.6	0.58	0.40, 0.86	0.007	0.59	0.45, 0.78	<0.001	0.21	0.07, 0.62	0.005	<0.001	<0.001
CCL3	0.73	0.52, 1.02	0.067	1.35	1.15, 1.58	<0.001	1.62	1.45, 1.81	<0.001	2.21	1.50, 3.27	<0.001	<0.001	<0.001
DCN	0.86	0.48, 1.55	0.6	1.98	1.49, 2.64	<0.001	2.61	2.13, 3.20	<0.001	8.35	4.24, 16.4	<0.001	<0.001	<0.001
AGRP	0.81	0.56, 1.18	0.3	1.47	1.22, 1.77	<0.001	2.01	1.76, 2.29	<0.001	3.35	2.00, 5.63	<0.001	<0.001	<0.001
PD_L2	1.06	0.64, 1.75	0.8	1.46	1.13, 1.88	0.003	2.29	1.91, 2.74	<0.001	5.22	2.63, 10.4	<0.001	<0.001	<0.001
hOSCAR	1.03	0.51, 2.10	0.9	2.3	1.50, 3.52	<0.001	1.51	1.13, 2.01	0.005	15.5	3.98, 60.5	<0.001	<0.001	<0.001
CD4	0.95	0.56, 1.60	0.8	1.76	1.36, 2.27	<0.001	2.27	1.89, 2.73	<0.001	4.55	2.37, 8.72	<0.001	<0.001	<0.001
CLEC4G	0.88	0.54, 1.43	0.6	1.57	1.23, 2.00	<0.001	1.71	1.43, 2.03	<0.001	2.33	1.13, 4.82	0.022	<0.001	<0.001
IL6	0.91	0.74, 1.12	0.4	1.33	1.21, 1.45	<0.001	1.54	1.44, 1.64	<0.001	1.83	1.49, 2.25	<0.001	<0.001	<0.001
DPP10	0.9	0.60, 1.34	0.6	1.35	1.14, 1.60	<0.001	1.67	1.49, 1.88	<0.001	1.77	1.15, 2.73	0.009	<0.001	<0.001
CDSN	1.15	0.83, 1.61	0.4	1.24	1.05, 1.46	0.013	1.51	1.34, 1.70	<0.001	2.57	1.60, 4.11	<0.001	<0.001	<0.001
LILRB4	0.91	0.65, 1.26	0.6	1.74	1.49, 2.04	<0.001	1.83	1.64, 2.05	<0.001	3.63	2.38, 5.51	<0.001	<0.001	<0.001
KRT19	1.14	0.88, 1.48	0.3	1.25	1.10, 1.42	<0.001	1.37	1.26, 1.50	<0.001	2.13	1.61, 2.81	<0.001	<0.001	<0.001
ITM2A	1.01	0.73, 1.41	0.9	1.21	1.02, 1.43	0.029	1.46	1.29, 1.65	<0.001	2.5	1.50, 4.16	<0.001	<0.001	<0.001
MILR1	0.93	0.63, 1.36	0.7	1.78	1.49, 2.12	<0.001	1.8	1.58, 2.04	<0.001	3.61	2.23, 5.85	<0.001	<0.001	<0.001
CD28	1.42	0.98, 2.06	0.067	1.33	1.09, 1.62	0.005	1.17	1.00, 1.36	0.044	1.65	1.01, 2.68	0.045	0.008	0.011
CKAP4	1.01	0.70, 1.44	0.9	1.72	1.46, 2.03	<0.001	1.91	1.70, 2.16	<0.001	3.13	2.16, 4.54	<0.001	<0.001	<0.001

Biomarker	Pulm Tissue congestion with HFpEF			Pulm tissue and Syst tissue congestion with AF			Pulm IV congestion with dilated LA & LV			Global congestion			P	P-overall	P-corrected
(Olink name)	OR	CI	p	OR	CI	p	OR	CI	p	OR	CI	p			
CLEC4D	0.92	0.69, 1.23	0.6	1.29	1.13, 1.48	<0.001	1.36	1.24, 1.50	<0.001	1.54	1.05, 2.27	0.028	<0.001	<0.001	
TREM1	0.89	0.61, 1.29	0.5	1.41	1.17, 1.70	<0.001	2.04	1.78, 2.34	<0.001	4.08	2.32, 7.16	<0.001	<0.001	<0.001	
CXADR	0.97	0.70, 1.36	0.9	1.41	1.20, 1.64	<0.001	1.96	1.76, 2.19	<0.001	3.15	2.15, 4.60	<0.001	<0.001	<0.001	
IL10	1.16	0.89, 1.51	0.3	1.38	1.23, 1.55	<0.001	1.32	1.21, 1.45	<0.001	1.43	1.05, 1.94	0.024	<0.001	<0.001	
NCR1	1.22	0.88, 1.71	0.2	1.4	1.19, 1.66	<0.001	1.16	1.03, 1.30	0.017	1.73	1.07, 2.79	0.026	<0.001	<0.001	
AREG	0.97	0.69, 1.35	0.8	1.56	1.34, 1.82	<0.001	2.03	1.82, 2.27	<0.001	2.96	2.05, 4.28	<0.001	<0.001	<0.001	
CLEC7A	1.01	0.77, 1.33	0.9	1.27	1.11, 1.46	<0.001	1.39	1.25, 1.53	<0.001	2.38	1.60, 3.54	<0.001	<0.001	<0.001	
CLEC6A	0.95	0.63, 1.41	0.8	1.54	1.28, 1.85	<0.001	1.39	1.22, 1.59	<0.001	1.82	1.09, 3.04	0.021	<0.001	<0.001	
IL12RB1	1.18	0.73, 1.89	0.5	1.49	1.18, 1.87	<0.001	1.53	1.30, 1.80	<0.001	2.04	1.11, 3.75	0.022	<0.001	<0.001	
ITGA11	1.36	0.88, 2.08	0.2	0.71	0.56, 0.89	0.003	0.63	0.54, 0.74	<0.001	0.37	0.19, 0.73	0.004	<0.001	<0.001	
LAG3	0.92	0.62, 1.38	0.7	1.38	1.15, 1.66	<0.001	1.46	1.28, 1.67	<0.001	2.09	1.34, 3.26	<0.001	<0.001	<0.001	
BTN3A2	1.11	0.75, 1.65	0.6	1.41	1.16, 1.72	<0.001	1.52	1.33, 1.75	<0.001	3.01	1.77, 5.11	<0.001	<0.001	<0.001	
VEGFA	0.84	0.55, 1.28	0.4	1.43	1.16, 1.75	<0.001	1.85	1.60, 2.14	<0.001	3.06	1.80, 5.21	<0.001	<0.001	<0.001	
TNFSF13	1.14	0.72, 1.80	0.6	2.15	1.69, 2.74	<0.001	2.73	2.29, 3.26	<0.001	8.26	4.01, 17.0	<0.001	<0.001	<0.001	
TNFRSF6B	1.07	0.82, 1.39	0.6	1.54	1.36, 1.74	<0.001	1.68	1.54, 1.84	<0.001	3.35	2.45, 4.60	<0.001	<0.001	<0.001	
IL6	0.91	0.75, 1.12	0.4	1.32	1.21, 1.43	<0.001	1.53	1.44, 1.63	<0.001	1.84	1.50, 2.26	<0.001	<0.001	<0.001	
CD48	1.15	0.62, 2.12	0.7	1.54	1.13, 2.09	0.006	1.42	1.14, 1.77	0.002	4.15	1.90, 9.07	<0.001	<0.001	<0.001	
LY9	1.04	0.63, 1.72	0.9	1.35	1.05, 1.74	0.018	1.67	1.40, 1.99	<0.001	3.43	1.74, 6.76	<0.001	<0.001	<0.001	
IFN-gamma-R1	1.14	0.66, 1.96	0.6	2.27	1.75, 2.96	<0.001	2.56	2.12, 3.10	<0.001	6.86	3.72, 12.7	<0.001	<0.001	<0.001	
TRAIL	0.64	0.38, 1.06	0.081	0.71	0.55, 0.92	0.009	0.42	0.35, 0.51	<0.001	0.24	0.13, 0.45	<0.001	<0.001	<0.001	
hK11	0.96	0.66, 1.41	0.9	1.23	1.02, 1.48	0.032	1.87	1.64, 2.13	<0.001	3.49	2.15, 5.67	<0.001	<0.001	<0.001	
TFPI-2	0.86	0.62, 1.20	0.4	1.56	1.35, 1.80	<0.001	1.86	1.68, 2.06	<0.001	1.76	1.19, 2.60	0.005	<0.001	<0.001	
VEGFR-2	1.16	0.56, 2.40	0.7	0.5	0.35, 0.70	<0.001	0.49	0.38, 0.63	<0.001	0.17	0.07, 0.39	<0.001	<0.001	<0.001	
TGF-alpha	0.79	0.49, 1.25	0.3	1.81	1.45, 2.26	<0.001	2.6	2.22, 3.05	<0.001	4.95	2.87, 8.55	<0.001	<0.001	<0.001	
EPHA2	1.4	0.93, 2.11	0.11	1.66	1.36, 2.03	<0.001	1.93	1.67, 2.23	<0.001	4.68	2.80, 7.84	<0.001	<0.001	<0.001	
ITGB5	1.04	0.59, 1.85	0.9	1.4	1.05, 1.86	0.021	1.82	1.49, 2.23	<0.001	3.4	1.64, 7.04	<0.001	<0.001	<0.001	
GPNMB	1.19	0.49, 2.91	0.7	2.06	1.30, 3.26	0.002	5.36	3.82, 7.54	<0.001	12	2.89, 49.5	<0.001	<0.001	<0.001	
CAIX	0.88	0.67, 1.16	0.4	1.29	1.13, 1.47	<0.001	1.54	1.41, 1.69	<0.001	1.94	1.37, 2.74	<0.001	<0.001	<0.001	
CD27	1.48	1.00, 2.20	0.051	1.27	1.04, 1.55	0.018	1.41	1.22, 1.62	<0.001	3.91	2.21, 6.92	<0.001	<0.001	<0.001	
ADAM 8	1.37	0.84, 2.23	0.2	1.37	1.08, 1.75	0.011	1.32	1.11, 1.56	0.002	3.08	1.54, 6.14	<0.001	<0.001	<0.001	
5'-NT	1.03	0.78, 1.36	0.9	1.43	1.25, 1.64	<0.001	1.73	1.57, 1.91	<0.001	2.35	1.55, 3.56	<0.001	<0.001	<0.001	
DLL1	1.45	0.86, 2.43	0.2	1.86	1.43, 2.42	<0.001	2.11	1.75, 2.54	<0.001	5.93	2.55, 13.8	<0.001	<0.001	<0.001	
VIM	0.76	0.55, 1.05	0.1	1.44	1.23, 1.68	<0.001	1.95	1.74, 2.18	<0.001	3.07	2.00, 4.72	<0.001	<0.001	<0.001	
TNFRSF19	1.12	0.85, 1.48	0.4	1.2	1.05, 1.38	0.009	1.46	1.33, 1.61	<0.001	2.65	1.88, 3.75	<0.001	<0.001	<0.001	
TCL1A	1.06	0.90, 1.25	0.5	0.88	0.81, 0.96	0.005	0.88	0.83, 0.94	<0.001	0.74	0.57, 0.96	0.025	<0.001	<0.001	
WISP-1	1.13	0.79, 1.61	0.5	2.04	1.72, 2.42	<0.001	2.75	2.43, 3.11	<0.001	5.85	3.76, 9.10	<0.001	<0.001	<0.001	
CXL17	0.82	0.59, 1.14	0.2	1.22	1.03, 1.45	0.019	1.46	1.30, 1.65	<0.001	2.65	1.69, 4.14	<0.001	<0.001	<0.001	
PPY	1.02	0.85, 1.21	0.8	1.13	1.03, 1.23	0.01	1.26	1.18, 1.35	<0.001	1.4	1.04, 1.87	0.025	<0.001	<0.001	
AREG	0.98	0.68, 1.42	0.9	1.59	1.35, 1.89	<0.001	2.09	1.86, 2.36	<0.001	3.3	2.24, 4.84	<0.001	<0.001	<0.001	
WFDC2	1.18	0.74, 1.87	0.5	1.62	1.27, 2.05	<0.001	2.73	2.28, 3.26	<0.001	8.17	3.81, 17.5	<0.001	<0.001	<0.001	
CXCL13	0.97	0.73, 1.28	0.8	1.34	1.18, 1.52	<0.001	1.42	1.30, 1.55	<0.001	2.05	1.50, 2.80	<0.001	<0.001	<0.001	
CD70	1.36	0.95, 1.96	0.093	1.66	1.39, 1.99	<0.001	1.19	1.04, 1.36	0.011	2.18	1.41, 3.37	<0.001	<0.001	<0.001	
RSPO3	1.07	0.86, 1.35	0.5	1.33	1.20, 1.48	<0.001	1.41	1.31, 1.52	<0.001	1.95	1.56, 2.45	<0.001	<0.001	<0.001	
FCRLB	0.76	0.54, 1.07	0.12	1.38	1.21, 1.57	<0.001	1.57	1.43, 1.73	<0.001	2.1	1.65, 2.69	<0.001	<0.001	<0.001	
TLT_2	1.52	1.15, 2.02	0.003	1.06	0.91, 1.22	0.5	0.74	0.67, 0.82	<0.001	1.64	1.08, 2.47	0.02	<0.001	<0.001	
T_PA	0.8	0.66, 0.96	0.02	1.07	0.99, 1.17	0.1	1.14	1.07, 1.20	<0.001	1.35	1.08, 1.68	0.008	<0.001	<0.001	
NT_pro_BNP	0.71	0.58, 0.86	<0.001	0.92	0.85, 1.01	0.077	1.81	1.70, 1.92	<0.001	2.27	1.84, 2.80	<0.001	<0.001	<0.001	
WIF-1	0.61	0.40, 0.93	0.023	1.21	0.98, 1.49	0.074	2.41	2.08, 2.80	<0.001	3.19	1.79, 5.69	<0.001	<0.001	<0.001	
EPHB4	1.61	1.15, 2.26	0.006	1.44	1.20, 1.71	<0.001	1.11	0.98, 1.27	0.1	3.11	2.07, 4.65	<0.001	<0.001	<0.001	
ALCAM	1.56	1.10, 2.22	0.012	1.4	1.17, 1.67	<0.001	1	0.88, 1.12	0.9	2.46	1.53, 3.95	<0.001	<0.001	<0.001	
MCP_1	1.48	1.12, 1.94	0.005	1.2	1.03, 1.41	0.021	0.96	0.85, 1.07	0.4	1.7	1.21, 2.39	0.002	<0.001	<0.001	
IL_18BP	1.46	1.10, 1.94	0.008	1.4	1.22, 1.62	<0.001	1	0.91, 1.10	0.9	2.86	1.91, 4.28	<0.001	<0.001	<0.001	
pdgf_subunit_a	0.61	0.48, 0.79	<0.001	0.81	0.72, 0.90	<0.001	1.01	0.94, 1.08	0.8	0.57	0.39, 0.84	0.004	<0.001	<0.001	
tnfrsf14	1.33	1.02, 1.72	0.033	1.26	1.10, 1.43	<0.001	1.15	1.05, 1.26	0.003	2.58	1.84, 3.62	<0.001	<0.001	<0.001	
tnf_r2	1.36	1.07, 1.73	0.013	1.43	1.27, 1.62	<0.001	1.19	1.09, 1.30	<0.001	2.66	1.96, 3.61	<0.001	<0.001	<0.001	
il2_ra	1.37	1.06, 1.77	0.017	1.33	1.16, 1.51	<0.001	1.11	1.01, 1.22	0.028	2.39	1.72, 3.31	<0.001	<0.001	<0.001	
tff3	1.27	1.01, 1.60	0.045	1.35	1.21, 1.52	<0.001	1.33	1.22, 1.44	<0.001	2.49	1.87, 3.30	<0.001	<0.001	<0.001	
gal_3	1.48	1.08, 2.03	0.014	1.2	1.02, 1.40	0.027	0.81	0.72, 0.90	<0.001	1.98	1.27, 3.08	0.003	<0.001	<0.001	
ltbr	1.43	1.07, 1.89	0.015	1.47	1.28, 1.70	<0.001	1.22	1.10, 1.35	<0.001	3.37	2.33, 4.88	<0.001	<0.001	<0.001	
notch_3	1.52	1.14, 2.04	0.005	1.52	1.31, 1.76	<0.001	1.3	1.17, 1.45	<0.001	2.91	2.06, 4.11	<0.001	<0.001	<0.001	
cntn1	1.54	1.12, 2.11	0.008	1.18	1.00, 1.39	0.047	0.81	0.73, 0.91	<0.001	1.82	1.16, 2.87	0.01	<0.001	<0.001	
fabp4	1.27	1.07, 1.51	0.006	1.51	1.38, 1.64	<0.001	1.32	1.24, 1.40	<0.001	1.88	1.48, 2.39	<0.001	<0.001	<0.001	
dlk_1	1.46	1.17, 1.82	<0.001	1.18	1.06, 1.32	0.003	0.88	0.81, 0.95	<0.001	1.88	1.36, 2.58	<0.001	<0.001	<0.001	
il_1rt1	1.42	1.03, 1.95	0.033	1.3	1.11, 1.53	<0.001	1.26	1.13, 1.41	<0.001	3.14	2.05, 4.80	<0.001	<0.001	<0.001	
mb	1.24	1.01, 1.52	0.036	1.16	1.04, 1.29	0.005	1.09	1.01, 1.17	0.022	1.7	1.30, 2.21	<0.001	<0.001	<0.001	
chi3l1	1.3	1.07, 1.56	0.007	1.29	1.17, 1.42	<0.001	1.11	1.04, 1.18	0.002	2.26	1.66, 3.09	<0.001	<0.001	<0.001	
col1a1	1.48	1.06, 2.06	0.021	1.43	1.21, 1.69	<0.001	1.16	1.03, 1.30	0.013	3.59	2.16, 5.95	<0.001	<0.001	<0.001	
ctsz	1.42	1.04, 1.96	0.03	1.33	1.13, 1.56	<0.001	0.86	0.78, 0.96	0.005	2.19	1.36, 3.54	<0.001	<0.001	<0.001	
vwf	0.78	0.65, 0.94	0.009	1.12	1.02, 1.22	0.013	1.09	1.03, 1.16	0.005	1.35	1.05, 1.73	0.02	<0.001	<0.001	
BNP	0.74	0.65, 0.84	<0.001	0.9	0.84, 0.96	<0.001	1.58	1.50, 1.66	<0.001	1.79	1.45, 2.21	<0.001	<0.001	<0.001	
CTSL1	0.56	0.36, 0.88	0.011	1.95	1.56, 2.43	<0.001	2.85	2.43, 3.35	<0.001	5.33	2.96, 9.58	<0.001	<0.001	<0.001	
LEP	1.55	1.28, 1.88	<0.001	1.33	1.21, 1.46	<0.001	0.77	0.72, 0.81	<0.001	0.75	0.60, 0.94	0.013	<0.001	<0.001	

Biomarker	Pulm Tissue congestion with HFpEF			Pulm tissue and Syst tissue congestion with AF			Pulm IV congestion with dilated LA & LV			Global congestion			P overall	P-corrected
(Olink name)	OR	CI	p	OR	CI	p	OR	CI	p	OR	CI	p		
SYND1	0.61	0.44, 0.84	0.003	1.3	1.11, 1.52	<0.001	2.21	1.97, 2.47	<0.001	2.19	1.39, 3.43	<0.001	<0.001	<0.001
TGFR-2	1.76	1.17, 2.65	0.006	1.74	1.42, 2.14	<0.001	1.47	1.27, 1.70	<0.001	4.31	2.39, 7.76	<0.001	<0.001	<0.001
HGF	0.71	0.52, 0.98	0.035	1.36	1.20, 1.53	<0.001	1.7	1.56, 1.85	<0.001	1.85	1.36, 2.50	<0.001	<0.001	<0.001
MK	0.77	0.60, 0.98	0.037	1.19	1.07, 1.33	<0.001	1.42	1.32, 1.53	<0.001	1.5	1.12, 2.01	0.007	<0.001	<0.001
ESM-1	0.64	0.46, 0.90	0.009	1.47	1.25, 1.73	<0.001	2.3	2.04, 2.59	<0.001	3.21	1.97, 5.25	<0.001	<0.001	<0.001
MUC-16	0.79	0.67, 0.92	0.002	1.23	1.15, 1.32	<0.001	1.52	1.44, 1.60	<0.001	2.48	1.92, 3.20	<0.001	<0.001	<0.001

Table 3S: Association of identified phenotype with composite outcome in the Nancy-HF after adjustment for clinical variable and treatment.

Phenotype	N events /N	Nancy-HF	
		HR (95% CI)	p-value
Model 2 + treatment			
Pulm-tissue congestion with dilated LV		Ref	
Pulm Tissue congestion with HFpEF		1.14 (0.66 - 1.96)	0.64
Pulm tissue and Syst tissue congestion with AF		1.70 (1.10 - 2.64)	0.018
Pulm IV congestion with dilated LA & LV		1.16 (0.76 - 1.77)	0.48
Global congestion		2.39 (1.57 - 3.64)	<0.0001

Reference: Pulm-tissue congestion with dilated LV
Model 1: adjusted for age, sex, BMI, SBP, LVEF, eGFR.
Model 2: Model M1 + NT-proBNP z score
The outcome was a composite of re-hospitalization for HF or death in the derivation cohort (Nancy-HF) and a composite of death and hospitalization in validation cohort (BIOSTAT-CHF).

4.3.1.1. Expanding the learning horizons

In this study, we concomitantly analyzed multiple congestion parameters from different domains to study the congestion profiles in patients with acute AHF. **Compared to the previous analysis conducted during this thesis, the novelty of this work is that it is focused on defining congestion profiles using an array of congestion markers**. This is one of the initial studies that assessed congestion profiles in HF using ML-driven models and validated the model in external cohort. **One of the principal challenges of this work was to learn to conduct clustering analysis and find stable and clinically meaningful clusters. Since, in unsupervised machine learning ground truth is unknown which makes interpretation of the identified clusters difficult**. For this analysis I realized clustering analysis and several techniques of supervised machine learning such as random forest, decision tree, and eXGBoost models to develop prediction models. Further, I learned to conduct multinomial logistic models to assess the association between clusters and protein biomarkers.

Adhering to the proposed framework for cluster analysis, we systematically executed all the steps to develop a validated model in this study.

Cluster identification: we incorporated all available parameters on systemic, pulmonary, tissue and vascular congestion sourced from clinical, echocardiographic and lab examination within our dataset (Nancy-HF) for this analysis. We selected the appropriate clustering model (LCM) for heterogenous data. The hyperparameters were tuned to obtain relatively stable phenotypes after clustering. LCM model apprised the discriminative power of each variable as well as less relevant variables to identify clusters. Interestingly, rales, eGFR and Hb were not identified to have significant discriminative power when profiling acute HF patients (In paper 2, it was also observed that number of B-lines did not differ according to eGFR status in hospitalized patients).

Internal validation: The internal validity of clustering was ascertained in the descriptive statistics, and also visually using radar plot to visualize the differences in characteristics and using KM curves and Cox models to visualize the survival differences.

Prediction model development and comparison: After identifying the clusters, we developed prediction models using several methods of supervised machine learning. The methods were compared on their ability to predict the cluster class in the derivation dataset.

Prediction model selection and Validation: Thereafter, the best-performing models were applied to the external cohort (BIOSTAT-CHF) for validation. The clusters derived in validation cohort were assessed for their congruity with the original clusters in the derivation cohort to identify best performing prediction model. The RF model was found to be most robust, producing clusters similar to derivation cohort in terms of clinical characteristics and prognosis.

Biomarker analysis: In addition, several protein biomarkers associated with each cluster were significantly different suggesting that each cluster potentially has distinct pathway associated with each cluster. This further validates our prediction model by showing that identified clusters were not only clinically different but also showed differences in protein biomarkers.

Our research laboratory has previously shown that clustering is an efficient way to identify individuals at higher risk of heart failure and predicting the associated risk in a population cohort (STANISLAS) [102]. In the aforementioned study, K-means clustering analysis was conducted on the echocardiographic parameters to identify homogenous echocardiographic phenotypes. Three different phenotypes were identified which had different levels of diastolic changes and structural remodeling as well as differed in their association with protein biomarkers. Subsequently, a decision tree was developed to predict the clusters which was validated in an external cohort (Malmo preventive Cohort). The validation cohort showed that these clusters differed in their risk of cardiovascular outcome.

Our findings underscore the utility of clustering analysis as a powerful method for identifying distinct homogenous and meaningful profiles within a heterogenous condition. The profiles were shown to differ significantly in their clinical characteristics as well as long-term outcomes. More importantly, it highlights the strength of clustering algorithms to facilitate a deeper understanding and exploration of the complex underlying pathophysiology.

5. Discussion

In this research work, we found that pulmonary congestion assessed through LUS is associated with poorer prognosis, regardless of age and LVEF in different clinical settings. Furthermore, degree of de-congestion, quantified by in-hospital changes in clinical congestion score, NP levels and B-lines, can improve risk stratification in HF regardless of LVEF. We also demonstrate that combination of pulmonary and systemic congestion was associated with poorer outcome than either sign of congestion alone. Through clustering analysis, we identified 5 distinct congestion phenotypes in HF by integrating various congestion parameters which were pulmonary tissue, systemic tissue, pulmonary intravascular, and systemic intravascular congestion. These phenotypes differ significantly in their underlying biological mechanisms and prognosis. Overall, our findings suggest that comprehensive congestion assessment is associated with better risk assessment and stratification than relying on few congestion parameters. A clustering analysis based approach appears promising for identifying congestion-based phenotype of HF. Furthermore, network analysis of protein biomarkers elucidated pathways associated with each congestion phenotype, thereby expanding our current understanding of their underlying pathophysiological mechanisms (**Central Figure**).

5.1. Identification of congestion profiles in HF

Through clustering, 5 different congestion profiles were identified which differed significantly in congestion signs and symptoms, NP levels and echocardiographic parameters despite some overlap of specific congestion parameters between phenogroups. These findings suggest that unsupervised machine-learning driven approach has the potential to detect more subtle and nuanced differences in HF patients than the conventional wet and dry classification. A previous study in acute HF patients reported that clusters derived from machine learning were associated with outcomes, but the identified profiles did not have clear relation with hemodynamic profile [148].

Individual congestion parameters and combined congestion score have been used to study congestion severity and its prognostic relevance. However, a more comprehensive picture emerges from an integrated analysis of different congestion parameters. For instance, in this study we identified three different (Pulmonary tissue congestion with HFpEF, Pulmonary tissue and Systemic tissue congestion with AF and Global congestion) where a large proportion of population had HFpEF (98%, 67% and 85% respectively) and nearly 90% population had rales yet, these profiles differed in presence of peripheral oedema, severity of LA dilation and PASP. These differences are not always obvious in conventional clinical assessment, especially for less experienced clinicians.

Unlike the previous studies which reported HF phenotypes, our primary focus in this study was to delineate congestion profiles. Our study provides the data-driven proof of conceptual classification of congestion as pulmonary/ systemic and tissue/ intravascular type. The concept proposed by Boorsma et al., and de la Espriella et al., is anchored in the current understanding of pathophysiology of congestion [3,8]. Our clustering analysis, performed on the real-world observational data, further supports the concept that congestion pattern, distribution and severity are indeed linked to distinct congestion profiles which have different clinical outcomes. The importance of identifying profiles lies in the fact that these profiles may be the "key" to understand underlying biological pathways for each phenotype and developing effective treatment.

5.1.1. Differences in biological pathways and response to treatment

In our study we observed that distinct phenotypes were associated with different protein pathways. While the immune response pathway was linked to all phenotypes, 'PTC-HFpEF' phenotype was related to lipid, protein and angiotensin metabolism and 'PTC, STC-AF' was related to kinase-mediated signaling, extracellular matrix organization and TNF-regulated cell death. Lastly, 'PIVC-dilated LA & LV' phenotype was related to kinase-mediated signaling and hemostasis. Similar findings have been

reported in previous studies where machine learning derived HF phenotypes were associated with specific proteins and pathways [149,150]. While the studies that explored the biological pathways of congestion profile remain limited, several studies have reported differential protein expression according to comorbidity profile such as obesity, AF, metabolic syndrome, ischemic cardiomyopathy and LVEF [151-154]. Our findings are in line with previously published literature which shows that patients with HFpEF, obesity and / or metabolic syndrome have immune/inflammatory pathways dysregulation whereas patients with HFrEF or ischemic-HF were associated with pathways that regulate endothelial dysfunction, coagulation, cardiac stretch and remodeling. Severe congestion on combined congestion score was associated with biological pathways of inflammation, endothelial activation, and mechanical stress in a previous network analysis in BIOSTAT-CHF cohort [11]. Similar findings were reported by Hedman et al., in the Karolinska- Rennes study where clustering derived phenogroups had distinct comorbidity profile, echocardiographic markers and protein biomarkers [149].

Additionally, previous studies indicate that unsupervised machine learning-based phenotyping can identify patient subgroups with varying treatment responses to beta-blockers and spironolactone [150,155-157]. In a pooled cohort data of 9 placebo-controlled trials of beta-blockers in HFrEF, 5 clusters were identified using neural network-based variational autoencoders and hierarchical clustering. Among the 5 identified clusters of patients in sinus rhythm, 4 clusters demonstrated a consistent benefit with beta-blocker treatment; however, beta- blocker therapy had sub-optimal efficacy in one cluster consisting of older patients and less severe symptoms [157]. On the contrary in patients with AF, 4 of the 5 phenogroups had neutral response to beta-blockers but one cluster consisting of young AF patients showed reduced mortality risk. However, the findings reported on the treatment effect of spironolactone in HF phenotypes were inconsistent. In a post hoc analysis of HFpEF patients enrolled in TOPCAT trial, latent class analysis identified three different clusters where phenogroup 1 was the youngest with low comorbidity burden [155]. Spironolactone was associated with significantly reduced risk of primary outcome of cardiovascular mortality, aborted cardiac arrest, or hospitalization for HF in the phenogroup 1 (low comorbidity burden)

[155]. Similar results were also reported in patients with ischemic HFrEF in post hoc analysis of EPHESUS and CAPRICORN trials, wherein MRAs were associated with higher risk reduction in-patients with lower comorbidity [158]. Whereas, in another analysis in TOPCAT trial, spironolactone therapy was associated with risk reduction in the cluster having higher comorbidity burden [150]. These differences in treatment response to spironolactone could have resulted from differences in methodology and variable selection between the two studies. In our analysis, we did not specifically test the response to treatment in identified phenotypes. However, the overall risk for outcome did not change when the Cox models were adjusted for medication use.

5.1.2. Translation into clinical practice

Despite the obvious advantages of machine learning approaches to identify homogenous groups within the complex heterogenous HF populations, the practical application in clinical practice remains challenging. Most of the studies lack validation in internal and external cohort hence, the results may not be generalizable in different populations. Further, only a few studies have published trained models which can be validated in external datasets. Another important factor challenging the applicability of machine learning models in clinical practice is that ML-driven models work as “black box” making result interpretation difficult. Moreover, legal and regulatory guidelines need to be established on data privacy and sharing.

Studies published till now are hypothesis generating because of heterogeneity observed in identified clusters. Consensus framework on types of models to be used, variables selection and interpretability along with user-friendly models that can be integrated in clinical practice could improve the acceptability of AI- driven model.

5.2. ML- models and their strength and weaknesses

In this study, we employed both unsupervised and supervised models to identify congestion phenotypes and subsequently developed model for assigning clusters in a validation dataset. To classify acute HF patients, we used varselLCM package available in R which is a model-based clustering method. It can analyze data composed of continuous, integer and categorical variable [159]. varselLCM provides certain advantages over LCA and Hierarchical clustering methods (the two most frequently used cluster method to identify HF phenotypes [112]) when analyzing medical data. Firstly, varselLCM can analyze data composed of continuous, categorical and integer variables whereas, LCA conventionally requires categorical data. Therefore, continuous variable should be converted to categorical data using pre-determined cut-offs for clustering analysis with LCA. In addition, varselLCM can handle missing data within the model thus, eliminating the need for separate imputation step. Moreover, this package is capable of selecting relevant variables whereas, this is not the case with LCA and Hierarchical clustering.

We tested several supervised machine learning models which could predict the cluster designated class in our study. We found that RF and eXGBoost models outperformed SVM and decision tree in correctly classifying the cluster class. Unlike RF models, eXGBoost has high accuracy in prediction as it sequentially develops the tree and learns from preceding classifier error. However, it requires hypertuning of parameters and if the parameters are not tuned appropriately there is a risk of overfitting the model. Hence, the results may not be generalizable to external datasets.

Compared to conventional statistical methods such as linear and logistic regression, machine learning models offer several advantages when the objective is classification and/or pattern detection. These models can identify non-linear relationships and analyze multiple variables even in the presence of collinearity. Further, tree-based models can capture non-linear interaction effects between variables which can be difficult with conventional statistics. However, interpretability of results is limited with

machine learning models. Conventional statistics are more suited when model transparency and interpretation are needed [160].

5.3. Comprehensive congestion assessment for improved congestion management and stratified treatment approaches

In all 4 manuscripts included in this thesis, we show that a comprehensive, combined assessment of different parameters of congestion can improve risk prediction in HF. In the paper 1 we show that adding details on pulmonary congestion can improve net reclassification of risk scores whereas paper 2 shows that serial congestion assessment of both clinical, biological and pulmonary congestion identifies patients at higher risk. Similar results were reported in a post hoc analysis of RELAX-AHF2 trial where high composite congestion score (≥3 on day 5 after admission) was associated higher worse outcome [161]. Subsequently, in paper 3 we assessed the impact of combined assessment of pulmonary and intravascular congestion. Paper 3 emphasizes that pulmonary and intravascular congestion assessment are complimentary to each other for identifying patients at risk and quantifying the risk. In paper 3, patients with isolated pulmonary or venous congestion were at lower risk compared to patients who had moderate to severe pulmonary congestion with venous congestion. Our results are in line with another analysis conducted on multimodality assessment of congestion in the pooled cohort [34]. It was reported that combined assessment of IVC, B-lines and PASP on top of clinical congestion score improved risk prediction over 90 and 180 days. Lastly, in our 4th paper we show that congestion profiles identified using clustering analysis of multiple congestion parameters can identify distinct profiles with varying risk of outcomes.

5.4. Limitations

All the analysis in this thesis are conducted in the retrospective cohort data. Hence, all the findings must be considered as hypothesis generating.

LUS cohort is a pooled patient-level data from 8 distinct European cohorts and therefore, could have introduced potential biases due to differences in available baseline data, methods of variable assessment and patient selection. Moreover, the pooled patient data (1947 patients), was moderate in size after its subsequent sub-division (time of admission, LVEF category), which limits the generalizability of the results. In addition, most of the patients likely received loop diuretics at admission, prior to ultrasound assessment of congestion. This could have influenced the number of B-lines and IVC diameter measurements. We sought to include several clinical, biological and echocardiographic parameters of congestion to model, however, assessment of hepatic, renal or portal venous congestion was not available. Furthermore, variations in adjudication procedures for HF hospitalization in each centre could have introduced additional biases.

In NANCY-HF and BIOSTAT cohort, B-line count and IVC diameter were not available and data based on rales and JVD cannot reliably assess pulmonary tissue and venous congestion. In BIOSTAT cohort, variables like E/e', PAPS and TAPSE were unavailable. This could have influenced the cluster designation by RF model. It would have been clinically meaningful to compare the cluster driven classification with Forrester classification; however, we did not have data on perfusion status in Nancy-HF cohort. In this analysis, we could not analyze whether a cluster derived profiles also had variability in response to HF drugs.

5.5. Strengths

Through the analysis undertaken during this thesis, we consistently show that integrated assessment of congestion improves risk stratification. In addition, our findings support the theoretical concept of different types of congestion profiles have different underlying mechanisms. Our findings demonstrate that not only congestion severity but also the interplay of various congestion parameters influences patient outcomes. These findings strengthen the notion that comprehensive congestion assessment is

needed to understand the impact of congestion parameters. Moreover, the intraclass correlation of the assessors of the LUS cohort was 0.8 suggesting a good reproducibility of the B-lines assessment across centers.

Our analysis successfully identified distinct congestion profiles, varying in clinical presentation and prognosis, through the application of unsupervised machine learning. The RF model developed for cluster prediction demonstrated more than 77% accuracy. Moreover, we validated our model in BIOSTAT-CHF, a large external cohort of HF patients thus confirming the usefulness of our model. The developed model can also be accessed through the webpage at https://cic-p-nancy.fr/ai-cong-hf/. Within the BIOSTAT-CHF cohort, we utilized data on the expression of 363 proteins assessed through proximity extension analysis. This data enabled us to explore the protein pathways linked with congestion profiles in HF. To the best of our knowledge, this is the first study that comprehensively explores congestion profiles using a wide array of congestion parameters and investigates potential biological pathways related to each congestion profiles.

6. Perspectives

In summary, this thesis demonstrated that unsupervised machine learning can identify meaningful congestion profiles and prognostic impact of integrated congestion parameters. Furthermore, we explored the biological underpinnings associated with the congestion profile. Moving forward, these findings have broader implications on congestion assessment and management and highlight possible areas for future research (**Central Figure**).

6.1. Need for a comprehensive congestion assessment for accurate and precise models

To advance the development of more precise and accurate models for congestion phenotyping, detailed data including clinical, echocardiographic and biological congestion parameters needs to be gathered in both in-patients and outpatients. **Future studies designed to collect more granular data on venous congestion, organ congestion and detailed echocardiographic data will be valuable in developing more parsimonious models.** In addition, data on response to decongestive therapies, patient reported quality of life outcomes, and telemonitoring symptoms/findings can be included in the model building to obtain a more holistic understanding. Furthermore, data on physical activity and food habits, frailty and other socio-demographic aspects should be integrated to have a better overall view of congestion profile in patients with HF. Leveraging on AI-driven models would ultimately lead to identification of homogenous congestion phenogroups within heterogenous HF population. These profiles would be helpful in exploring the pathophysiology of specific groups, guiding personalized treatment strategies, and enhancing risk assessment and prognosis.

6.2. Semi -supervised learning models to improve prediction models

One of the principal challenges to developing AI driven models in healthcare settings is lack of labelled data. For instance, **annotation of medical imaging data like echocardiography or electrocardiogram is both time consuming and costly**. Further, inaccuracies in echocardiographic assessment can be up to 30% in physician reported echo depending on the training [162]. Hence, limiting the availability of useable data for analysis.

Semi- supervised machine learning methods is a hybrid method that combines small amounts of labelled data and large amount of unlabeled data for training. This combination of supervised and unsupervised learning can be used to predict and find patterns in vast amounts of un-labelled data. **Semi-supervised ML methods provide a balance between large high performance obtained with supervised labelled data analysis and moderate to good accuracy at lower time and cost required for data annotation**. A recent study showed that semi-supervised ML models trained on small labeled and large unlabeled data consisting of several still-image echocardiographic view could classify left ventricular hypertrophy with 80% accuracy [162] and predict AF using a small labelled electrocardiograph data[163]. Similarly, use of semi-supervised learning in a recent study identified acute HF patients phenogroups with distinct characteristics with a specific focus on low diuretic efficiency. Guided by this model researchers developed a risk prediction tool for low diuretic efficiency in patients with acute decompensated HF. Identifying sub-groups with different diuretic response could be a step towards the development of personalized decongestion approach [164].

Adopting semi-supervised ML approach can be a practical solution to obtain a generalizable model using larger, readily available dataset leveraging on fewer labelled data. This method can also be used to identify/specify diagnosis in datasets obtained through electronic health records.

6.3. Integrating with electronic health records

Identification of HF phenotypes remains restricted to academic research. Bridging the gaps between clinical application and in silico research calls for more comprehensive real-world data base. One of the potential solutions is use of **electronic health records to identify potential variables of interest** in the disease population. Moreover, **EHRs can provide longitudinal data which can be used to analyze temporal trends in changes in symptoms, lab results, and medications.** These metrics can be incorporated as variables in prediction models **to improve model performance**. However, extracting data from clinical notes to develop comprehensive EHR is challenging. Advancements in methods of data extraction such as natural language processing are needed to extract data points from clinical notes.

Advanced prediction models embedded in EHR database can assist in making real-time risk prediction and improving decision making for treatment individualization. By analyzing commonly assessed congestion variables, the AI-model can identify a patient's congestion profile, which is important for managing HF. The AI model developed during the thesis can identify the congestion profiles using commonly assessed congestion variables. **This online model has the potential to be integrated within the EHRs for routine clinical practice. Integrating EHRs and future risk prediction models can be clinically relevant for early identification and referral for more intensive treatment in HF population.**

6.4. Improving clinical interpretation of identified clusters

The clusters identified using unsupervised machine learning methods **require interpretation by domain experts,** as the models do not provide insight into how the clusters are different. Several methods can be incorporated in the study design to enhance the interpretability and clinical relevance of the clusters. While **descriptive statistics** are the foundational step to distinguish between clinical characteristics, **visualization techniques like heat map and radar plot** provide a quick overview of the

distinctive characteristics of the cluster. Several clustering algorithms inform relative **importance of variables** that drive the clustering. Importantly, these variables should be interpreted in light of their clinical relevance. In addition, a **simple decision tree** predicting cluster class demonstrates the clear and highly interpretable rules for characterizing and understanding clusters. Another important aspect that facilitates interpretation of identified clusters is labelling them with **meaningful labels** based on the predominant characteristics. Several studies report clusters in an alphanumeric manner (for example, cluster 1, cluster 2) which makes it difficult for readers to decipher the clinical context of the clusters rapidly and distinguish between the profiles.

The HF phenotypes identified through clustering analysis in various published articles have considerable similarities in clinical and demographic profiles. However, **significant heterogeneity also exists in these overlapping clusters** making it difficult to implement these profiles in regular clinical practice. Moreover, a patient is likely to fall into different cluster if different clustering algorithms developed in distinct datasets or using different methodology are applied to their data. Two systematic reviews have analyzed the studies identifying HF profiles using unsupervised machine learning [112,165]. Both these studies aimed to summarize the profiles broadly on demographic and comorbidity parameters. However, this approach has its limitations. For example, a patient with HF with AF may fall under different category depending on the presence / probability of other comorbidities. To some extent this issue can be addressed if the prediction model is shared with prospective users and **validated in external datasets**.

6.5. Validation and approval of models from regulatory authorities

To ensure the clinical utility and robustness of the model, it should be validated in external multicentric cohort. **Validation on an external dataset improves the generalizability of the model** as it demonstrates model reliably identifies the clusters and that findings are not a result of over-fitting.

More studies with robust methodology are needed to generate high level of evidence on cluster driven outcomes. To this end, we proposed a framework to develop and validate AI-driven prediction models in cardiovascular disease [166]. Similar **framework or consensus statements are required to enhance the quality of studies involving clustering analysis (Figure 9)**.

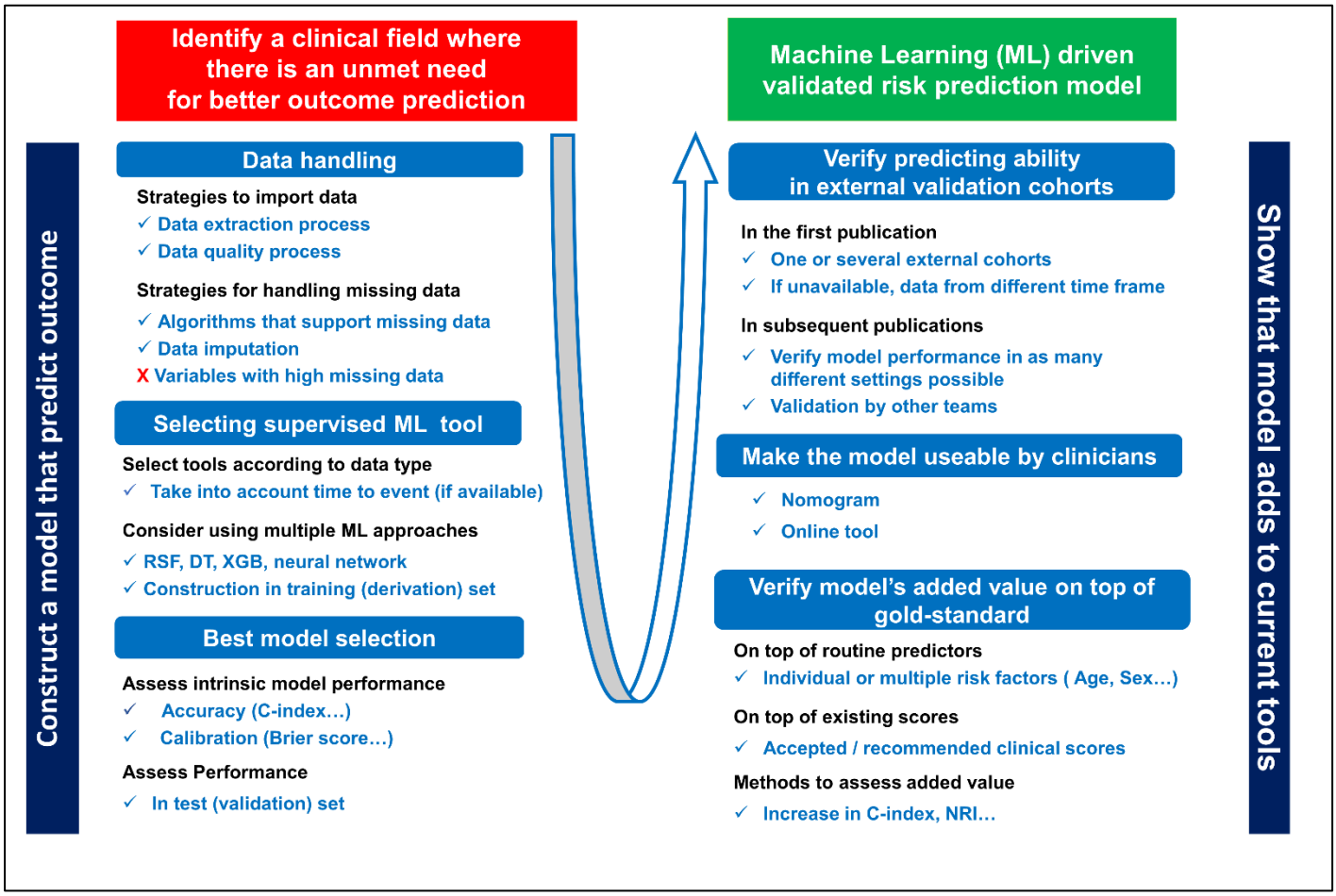


Figure 9: Framework to develop and validate AI-driven prediction models in cardiovascular disease (from Rastogi et al., [166]).

Incorporation into regular clinical practice will also require approval from regulatory authority. Several AI-models have been approved by Food and Drugs Authority, USA in field of radiology for image reading software however, such approved software are limited in the field of cardiology [167]. Although few medical devices and algorithms have been approved which detect cardiac

rhythm abnormalities. **Approval from regulatory authorities can assure credibility and accuracy of algorithms before they assist clinical decision making**.

6.6. Utility in guiding patient management

Most of the published studies have validated the distinctness of clusters by demonstrating different prognosis for each profile. Although this does provide an internal validity to identified profiles, it leaves out a more **important question of clinical utility of these profiles in guiding patient management**. Future studies should assess if the specific drugs improve risk of adverse outcomes in an identified cluster or not, especially in HFpEF population. For example, Karwath, et al., identified a HF profile amongst patients with HF and LVEF > 50% that had reduced risk with beta-blocker use [168]. This finding can be used to design a clinical study testing efficacy of beta-blockers in this specific subgroup. Such an approach would provide the ultimate proof of a data-driven model's clinical utility: demonstrating that it truly segments patients based on distinct underlying pathophysiology and identifies actionable phenotypes for targeted therapeutic intervention. Ultimately, treatment could be individualized according to the clusters.

However, what would constitute an effective management strategy in patients identified to be at higher risk still needs to be assessed in randomized clinical trials. This approach could be useful in titrating diuretics dose or selecting additional drugs especially in patients with residual congestion at discharge.

In this study we identified 5 different congestion clusters in the Nancy-HF cohort and validated the findings in an external cohort and demonstrated the difference in underlying pathophysiology. However, the utility of these clusters in testing de-congestive therapies in future studies needs to be ascertained. The **figure 10** below shows the predominant congestion type with identified clusters.

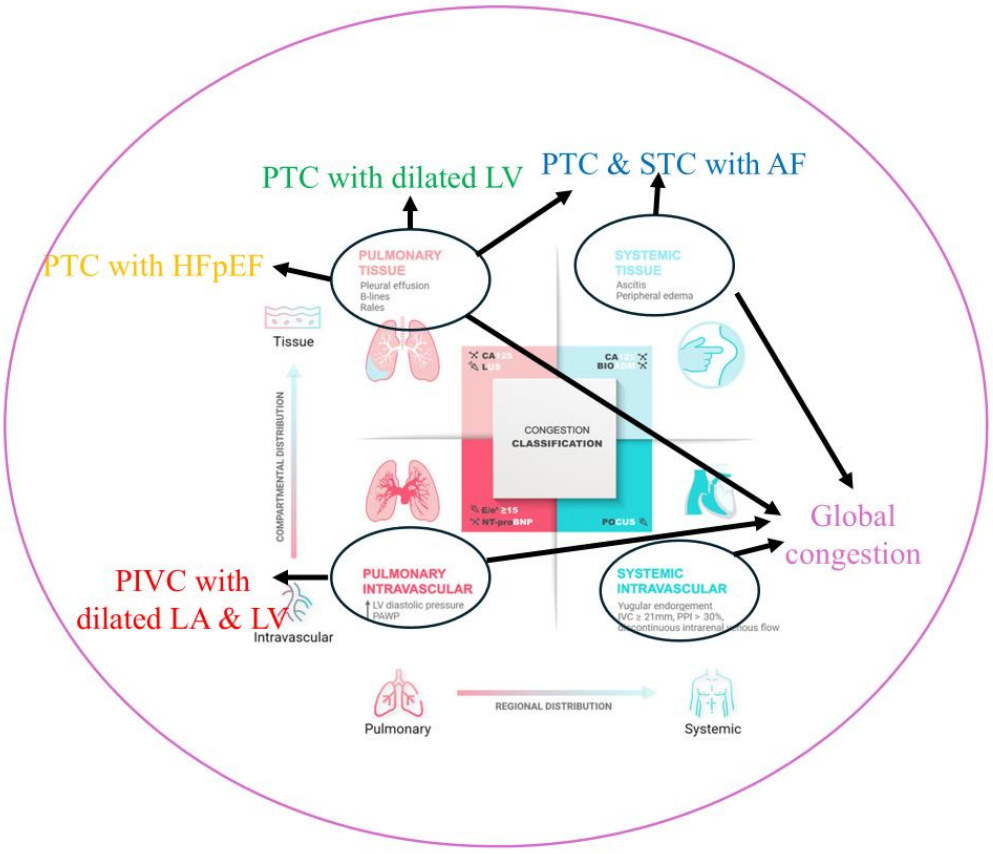


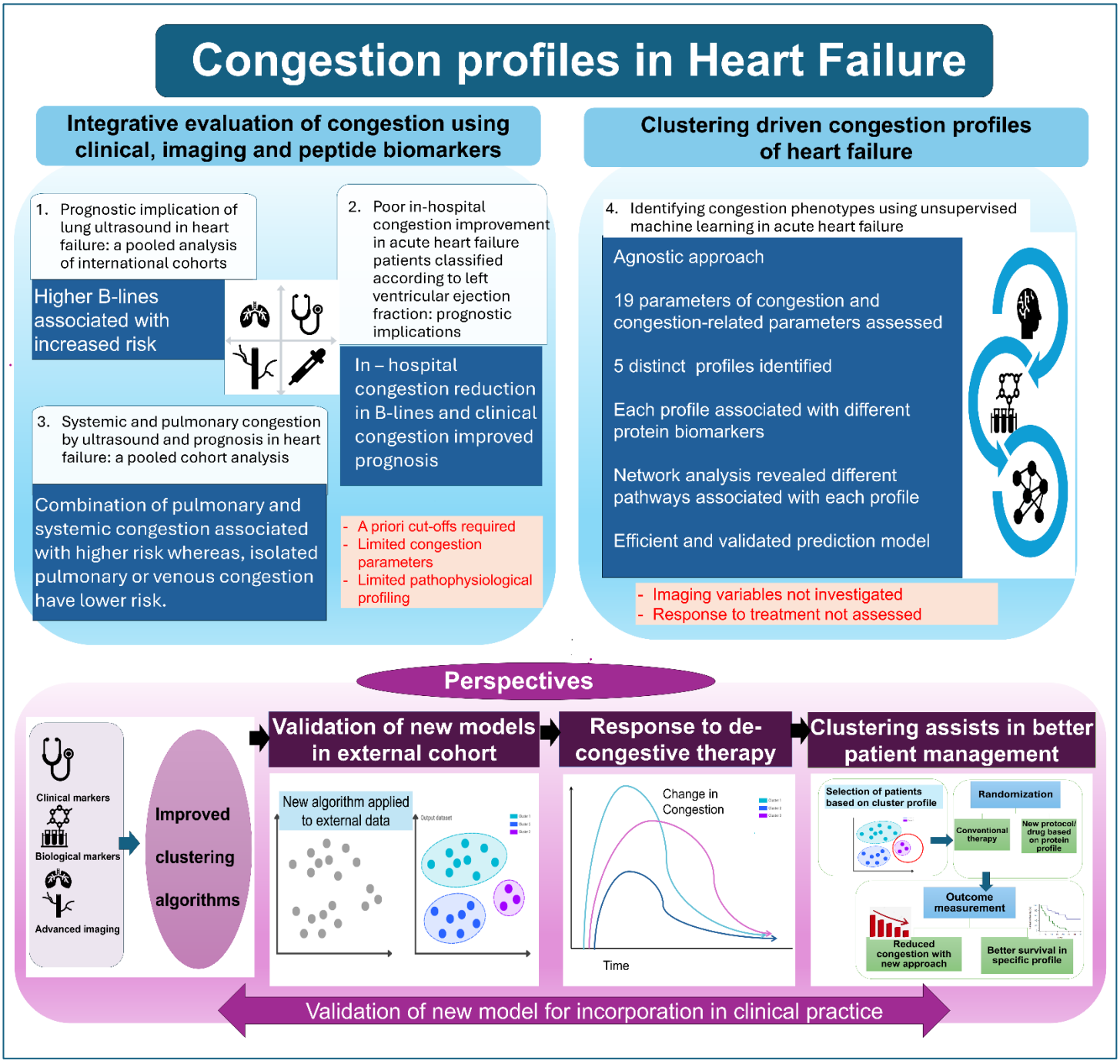
Figure 10: Congestion profiles identified through clustering and their predominant congestion subtype.

This figure shows the predominant congestion subtype that is pulmonary or systemic congestion that was observed in profiles identified through clustering. PTC with HFpEF and PTC with dilated LV had predominantly pulmonary tissue congestion whereas, PTC & STC with AF had both pulmonary tissue and systemic tissue congestion. PIVC with dilated LA & LV had predominantly pulmonary intravascular congestion. Global congestion phenotype had all 4 subtypes of congestion.

7. Conclusions

We conclude that a combined assessment of pulmonary, systemic, tissue, and intravascular congestion can improve risk stratification in patients with acute and chronic HF. Using clustering analysis, we identified 5 different congestion profiles, each having distinct congestion parameters and potentially different underlying mechanisms. Machine learning techniques are better suited to identify complex congestion patterns and profiles, given their ability to integrate large array of heterogenous data. However, additional research with strong methodological rigor is required before AI-driven models can be integrated in routine clinical practice.

Central figure



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Abstract (English version)

Congestion is the main driver of symptoms and hospitalization in heart failure (HF) and is linked to the prognosis. Accurate congestion assessment can provide information on disease severity, prognosis and guide treatment decisions. Currently, congestion is evaluated by several tools such as physical examination, natriuretic peptides (NP), echocardiography and lung ultrasound (LUS). However, the prognostic utility of LUS is not yet well-established in different clinical settings and patient profiles. It is also unclear whether combining LUS findings with clinical findings, NP and other echocardiographic parameters can improve risk stratification. A deeper understanding of congestion mechanisms, its pattern and distribution and their impact on prognosis would be vital for classifying HF and developing novel therapies. Conceptual framework classifying congestion into pulmonary, systemic, intravascular and tissue congestion have been proposed but lack robust evidence. Machine learning (ML) models that simultaneously analyze multiple congestion parameters can play a vital role in identifying congestion profiles and underlying mechanisms.

In this thesis, we assessed the prognostic value of a) pulmonary congestion evaluated by B-lines across clinical profiles and clinical setting, b) in-hospital changes in congestion either evaluated by clinical, imaging or natriuretic peptide, c) combined assessment of pulmonary (B-lines) and systemic congestion (IVC diameter) in HF. Additionally, we aimed to identify and characterize distinct congestion profiles in HF using clustering based on clinical, biological, and echocardiographic congestion variables and to assess their prognostic significance and underlying biological pathways via complex network analysis.

In this study we showed that a comprehensive congestion assessment improves risk stratification in HF. We found that higher B-line count was associated with increased risk of outcomes at discharge and in out-patients but not at admission. Moreover, prognostic capacity of B-lines was not influenced by age, sex, LVEF or presence of AF in the out-patients. When assessing in-hospital congestion changes, patients who showed no/limited improvement in diverse combinations of congestion parameters had varying adverse event rates. Also, presence of both pulmonary and systemic congestion was associated with worse prognosis whereas, isolated systemic and pulmonary congestion was less predictive of poorer outcomes.

Using ML models, we identified 5 different congestion profiles which were associated with different long-term risk. Network analysis revealed that each profile was associated with different proteins and biological pathways. We developed the random forest model (Ai-Cong-HF: https://cic-p-nancy.fr/ai-cong-hf/) which may be used to identify the congestion phenotype.

Our work shows that a combined assessment of pulmonary, systemic, tissue, and intravascular congestion can improve risk stratification in HF. Unsupervised ML techniques can be extremely useful to identify congestion profile and understand pathophysiological underpinnings. More studies with robust methodology are needed before ML models are integrated in clinical practice.

Keywords: heart failure, congestion, echocardiography, clustering, protein biomarker, complex network

Abstract (version français)

La congestion est le principal facteur responsable des symptômes et des hospitalisations dans les cas d'insuffisance cardiaque (IC) et est liée au pronostic. Une évaluation précise de la congestion peut fournir des informations sur la gravité de la maladie, le pronostic et orienter les décisions thérapeutiques. Actuellement, la congestion est évaluée à l'aide de plusieurs outils - l'examen physique, les peptides natriurétiques (PN), l'échocardiographie et l'échographie pulmonaire. Cependant, l'utilité pronostique de l'échographie pulmonaire n'est pas encore bien établie dans différents contextes cliniques et profils de patients. Il n'est pas clair si la combinaison des résultats de l'échographie pulmonaire avec les résultats cliniques, les PN et d'autres paramètres échocardiographiques peut améliorer la stratification des risques. Une meilleure compréhension des mécanismes de la congestion, de son schéma et de sa distribution, ainsi que de leur impact sur le pronostic, serait essentielle pour classer l'IC et développer de nouvelles thérapies. Un cadre conceptuel classant la congestion en congestion pulmonaire, systémique, intravasculaire et tissulaire a été proposé, mais il manque de preuves solides. Les modèles d'apprentissage automatique (ML) qui analysent simultanément plusieurs paramètres de congestion peuvent jouer un rôle essentiel dans l'identification des profils de congestion et des mécanismes sous-jacents.

Dans cette thèse, nous avons évalué la valeur pronostique a) de la congestion pulmonaire évaluée par les lignes B dans différents profils cliniques et contextes cliniques, b) des changements intra-hospitaliers de la congestion évalués par des examens cliniques, l'échographie pulmonaire ou par le peptide natriurétique, c) de l'évaluation combinée de la congestion pulmonaire et systémique dans IC. En outre, nous avons cherché à identifier et à caractériser différents profils de congestion à l'aide de clustering basé sur des variables cliniques, biologiques et échocardiographiques de congestion, et à évaluer leur signification pronostique et les voies biologiques sous-jacentes via une analyse de réseau complexe.

Dans cette étude, nous avons montré qu'une évaluation détaillée de la congestion améliore la stratification des risques dans l'IC. Nous avons constaté qu'un nombre élevé de lignes B était associé à un risque accru à la sortie de l'hôpital et chez les patients ambulatoires, mais pas à l'admission. De plus, la capacité pronostique des lignes B n'était pas influencée par l'âge, le sexe, la FEVG ou la présence d'une fibrillation auriculaire chez les patients ambulatoires. Lors de l'évaluation des changements de congestion à l'hôpital, les patients qui ne présentaient aucune amélioration ou une amélioration limitée dans diverses combinaisons de paramètres de congestion avaient des taux d'événements indésirables variables. De plus, la présence d'une congestion pulmonaire et systémique était associée à un pronostic plus défavorable, tandis que la congestion systémique et pulmonaire isolée était moins prédictive de mauvais événements cliniques.

À l'aide de clustering, nous avons identifié 5 profils de congestion différents associés à différents risques à long terme. L'analyse de type réseau complexe à révéler que chaque profil était associé à différentes protéines et voies biologiques. Nous avons développé le modèle Random Forest (Ai-Cong-HF : https://cic-p-nancy.fr/ai-cong-hf/) qui peut être utilisé pour identifier le phénotype de congestion.

Nos travaux montrent qu'une évaluation combinée de la congestion pulmonaire, systémique, tissulaire et intravasculaire peut améliorer la stratification des risques dans l'IC. Les techniques d'apprentissage automatique non-supervisée peuvent être très utiles pour identifier le profil de congestion et comprendre les mécanismes physiopathologiques. D'autres études utilisant une méthodologie robuste sont nécessaires avant que les modèles de ML soit intégré dans la pratique clinique.

Mots clés: insuffisance cardiaque, congestion, échographie cardiaque, clustering, biomarqueur protéique, réseau complexe