Multimarkers approach in emergency medicine

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Approche multimarqueurs en médecine d'urgence

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Thèse de doctorat

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« D’ailleurs j’ai purement passé les jours mauvais

Et je sais qui je suis si j’ignore où je vais. »

Sommaire

PREMIERE PARTIE :

Les biomarqueurs

aux urgences

I) Le diagnostic

Tout au long de ce travail, nous allons développer et évaluer quelques approches multimarqueurs aux urgences. Le principe des études que nous allons présenter et discuter est celui de toute étude diagnostique, voire toute étude scientifique.

Une étude clinique, comme toute expérience scientifique va chercher à répondre à une question, une question quantifiable. Selon Karl Popper, épistémologue du début du siècle dernier, la démarche scientifique doit procéder par rejet d'hypothèse. L'expérience doit en effet servir à justifier ou prouver notre hypothèse. Or vérifier la véracité d'une hypothèse lors d'une expérience ne peut en aucun cas être la démonstration de cette hypothèse. On s'explique : pour prouver une causalité « A => B », il ne suffit pas de constater A et B. Si l'on passe par la contraposée « (non B) => (non A) », l'expérience qui constaterait (non A) et (non B) ne prouverait rien non plus. En revanche, il est très facile de démontrer qu'une causalité n'existe pas.

Pour réfuter l'hypothèse « A => B », il suffit d'une expérience qui montre (non B) ET A.

Ceci va constituer la base théorique des recherches scientifiques et des études cliniques médicales. Une hypothèse est formulée (l'hypothèse nulle) et l'expérience, l'étude, va s'efforcer de la réfuter.

L'approche multimarqueur aux urgences va être ici évaluée, comme toute étude sur les biomarqueurs, en suivant la démarche plus générale des études diagnostiques. Le report de ces études est encadré par les règles STARD [1] (Standards for the reporting of diagnostic accuracy studies – http://www.stard-statement.org) qui sont composées entre autre de 25 items clés nécessaires à l'interprétation de toute étude diagnostique, et sans lesquels le risque de biais serait trop important et sous évalué.

En suivant ces règles, et en s'inspirant de la démarche préconisée par K Popper, nous allons évaluer l'intérêt de l'approche multimarqueur dans l'aide au diagnostic aux urgences. Cette approche sera réalisée aussi pour la prédiction de l'aggravation ou la stratification du risque sur le même modèle, ces deux notions n'étant pas tout à fait disjointes : par exemple, le diagnostic de sepsis sévère est un diagnostic à part entière, mais peut être considéré comme une strate du risque parmi les états septiques. Aussi, dans ce travail, nous adopterons la même attitude concernant les études diagnostiques que pour les études « pronostiques » ou visant à évaluer la stratification du risque.

II) Généralités sur les biomarqueurs

Un biomarqueur est un paramètre biologique dont la mesure peut apporter une information sur l'état de santé d'un sujet ou son évolution.

En 2001, le « Biomarkers Definitions Working Group » a défini un biomarqueur comme étant « une caractéristique qui peut être mesurée de manière objective, et être évaluée comme indicateur d'un processus physiologique, pathologique, ou encore d'une réponse à une intervention thérapeutique »[2]. Ainsi, la taille, le poids, la pression artérielle systolique, le VPS34-IN1, l'Interleukine-6 ou la couleur des cheveux sont donc des biomarqueurs. La mesure et l'étude des biomarqueurs recouvrent ainsi une grande variété d'applications, en particulier :

- Le diagnostic d'une pathologie : par exemple, la pression artérielle pour le diagnostic d'hypertension artérielle ou la troponine dans le syndrome coronaire aigu (SCA)

- La stratification de la gravité d'une maladie ou l'évaluation de son extension : par exemple, le lactate dans le sepsis, ou le Prostate Specific Antigen (PSA) dans le cancer de la prostate

- Le pronostic d'une pathologie : la taille d'une tumeur, la pression artérielle dans le sepsis…

- Et la prédiction d'une réponse ou la surveillance après intervention thérapeutique : l'agrégation plaquettaire avant introduction de clopidogrel ou le taux de cholestérol après introduction de statine.

De manière concomitant à la définition d'un biomarqueur, le groupe de travail a de même défini le concept de « critère de jugement clinique » (« clinical endpoint »), indispensable pour lier de manière rigoureuse la mesure du biomarqueur à l'état de santé d'un malade. Il est défini comme une caractéristique ou une variable qui reflète l'état sensoriel du patient (douleur, dyspnée), fonctionnel (handicap, force musculaire), ou sa survie. Enfin, sont définis les « surrogate endpoints » que l'on pourrait traduire par critères de jugement de substitutions : dans certains cas bien encadrés, la valeur d'un biomarqueur pourrait se substituer à un critère de jugement clinique [3].

Les biomarqueurs actuellement utilisés ou développés sont le plus souvent des protéines ou molécules dosable dans le sang ou les urines. Le développement d'un nouveau biomarqueur doit suivre un processus en cinq étapes qu'on peut caler sur celui recommandé par le Early Detection Research Network (EDRN) du National Cancer Institute aux USA [4]. Celui-ci est composé de :

- Une phase de test pré-clinique de comparaison entre tissu (ou population) sain, et tissu (ou population) pathologique. Le but est d'identifier des candidats biomarqueurs dont la mesure serait différente entre individus sains et malades.

- Suivie d'une phase de développement d'un test reproductible. Son objectif principal est d'estimer le taux de vrai positifs et faux positifs dans un échantillon représentatif de la population – la qualité « cas » ou « contrôle » étant déjà connue.

- Une étude rétrospective sur une population malade pour confirmer l'intérêt potentiel du biomarqueur : une positivité (ou anomalie) de la mesure du biomarqueur doit précéder le développement clinique de la maladie.

- Une phase d'étude prospective sur une population cible afin de déterminer les performances et l'utilité théorique du biomarqueur

- Enfin, une étude d'impact qui démontrerait que l'utilisation du biomarqueur en pratique clinique a un intérêt clinique, économique ou autre.

III) Evaluation d'un biomarqueur

On évoquera ici très brièvement les principes de bases et avancées de l'évaluation statistique des biomarqueurs. Les performances statistiques sont dérivées et extrapolées à partir d'études cliniques réalisées dans ce but. Il est donc primordial que la méthodologie soit d'une rigueur extrême et réponde à un processus standardisé afin de pouvoir se fier aux résultats. Ainsi, les recommandations internationale STARD (Standard for Reporting of Diagnostic Accuracy) ont été

élaborées et servent de base au report et à l'analyse d'études diagnostiques [1]. Elles définissent un cadre pour la conception, la réalisation et le rapport des études diagnostiques. Elles insistent en particulier sur la nécessité de bien décrire la population cible (les critères d'inclusion et de non inclusion, la sélection des patients incluables), les méthodes de classification (méthode de référence, entrainement des experts), la nécessité d'expliciter un tableau de contingence ou encore des règles sur l'analyse statistique.

L'étape première et indispensable pour de telles analyses et interprétations est la création d'un tableau de contingence, classant les individus selon la méthode de référence et selon le biomarqueur testé :

	Maladie	
Biomarqueur	Malade	Sain
Positif	a (VP)	b (FP)
Négatif	c (FN)	d (VN)

Table 1 : Tableau de contingence type.
VP : vrai positif, FP : faux positif, FN : faux négatif, VN : vrai négatif.

De ce tableau découle immédiatement une estimation des qualités basiques du biomarqueur étudié qu'on rappellera pour la forme :

Sensibilité	*Probabilité qu'un malade ait un résultat positif*	a/(a+c)
Spécificité	*Probabilité qu'un non malade ait un résultat négatif*	d/(b+d)
Valeur Prédictive Négative	*Probabiltié qu'un résultat négatif corresponde à un non malade*	a/(a+b)
Valeur Prédictive Positive	*Probabilité qu'un résultat positif corresponde à un malade*	d/(c+d)

Afin d'affiner les performances du biomarqueur, en suivant l'approche Bayesienne, on peut évaluer initialement la probabilité pré-test pour ensuite calculer la probabilité post test après mesure du biomarqueur. Pour ce faire on calcule les rapports de vraisemblances positifs (RV+) et négatifs (RV-) (RV+=Se/(1-Spe) et RV-=(1-Se)/Spe) et les reporte sur le nomogramme de Fagan [5].

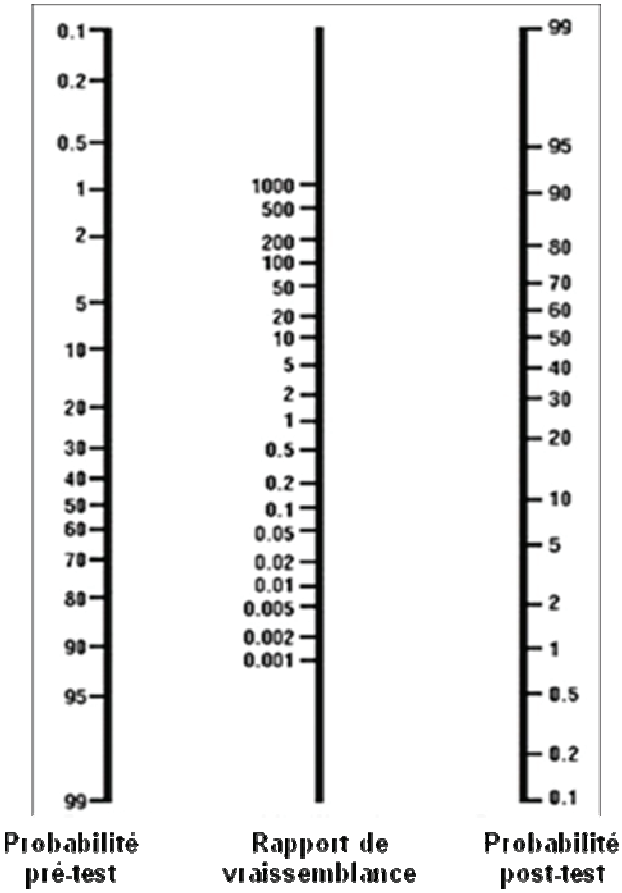

Figure 1 : Nomogramme de Fagan

Nous allons illustrer cette approche avec le cas du diagnostic d'insuffisance cardiaque aiguë et la mesure du Brain Natriuretic Peptid (BNP). Korenstein et al. ont réalisé une revue systématique en 2007 qui donne une valeur agglomérée des

caractéristiques diagnostiques du BNP, dont ils tirent les RV positifs et négatifs. Ainsi, pour un seuil à 100 pg/ml, les auteurs rapportent un RV+=3.4 et un RV- =0.14. Pour comprendre la portée de ces valeurs, nous les reportons sur le nomogramme pour 3 cas de figures : une probabilité pré-test à 10%, 50% et 90% :

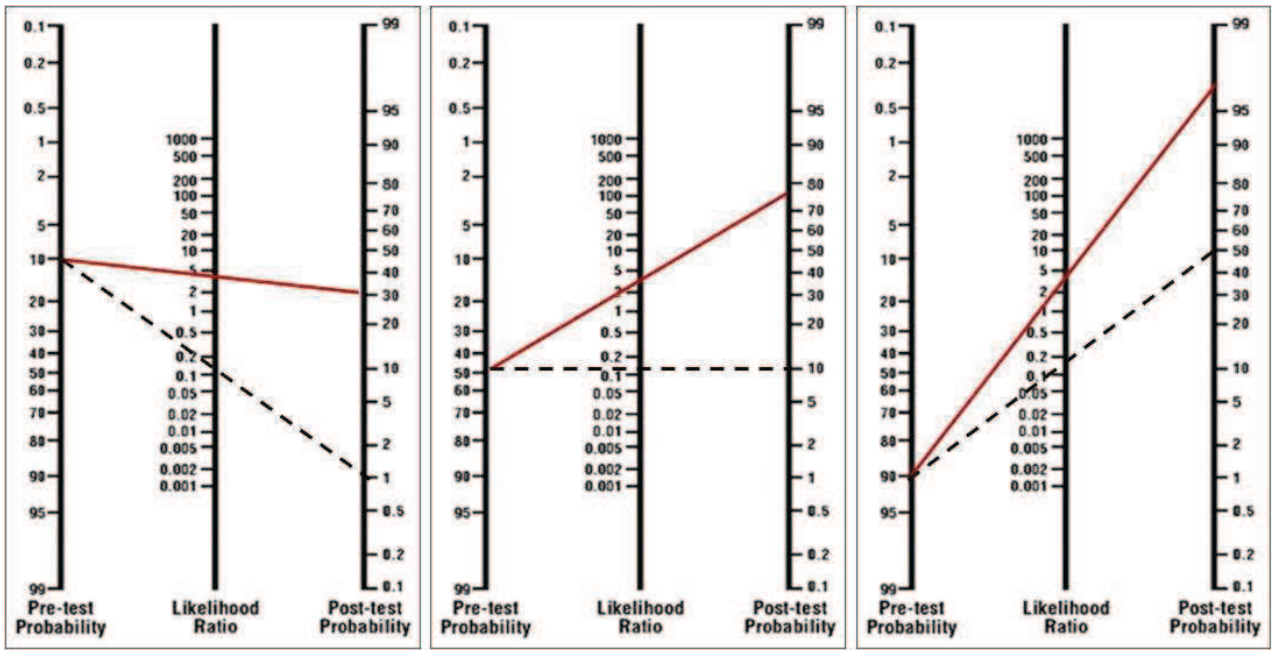

Figure 2 : Nomogramme de Fagan. Ligne continue : rapport de vraisemblance positif. Ligne pointillée : rapport de vraisemblance négatif

Pour le premier cas, en cas de résultat positif la probabilité passe de 10% à 30%, et de 10% à 1% en cas de résultat négatif. L'apport du dosage de BNP dans ce cas là est donc très limité, le résultat ne modifiera probablement pas les hypothèses diagnostiques et la prise en charge. Le raisonnement est identique à l'inverse pour le troisième cas, avec une probabilité pré-test forte.
En revanche, l'apport du BNP est bien illustré sur le deuxième cas. Selon le résultat du dosage de notre biomarqueur, on passe d'une probabilité intermédiaire à une probabilité forte (si positif) ou faible (si négatif) d'insuffisance cardiaque aiguë.

Selon l'utilisation attendue du biomarqueur, différentes performances seront recherchées. Par exemple si l'on cherche à évaluer un marqueur diagnostique du syndrome coronaire aigu, une excellente sensibilité ou valeur prédictive négative sera recherchée afin de limiter le risque de faux négatifs. Pour un dépistage de masse de maladie grave (VIH, cancer), de même, on cherchera un marqueur avec une grande sensibilité. En revanche, pour confirmer une maladie grave, avant d'entreprendre des traitements lourds et couteux, on cherchera à confirmer le diagnostic par un test d'une sensibilité extrême, pour réduire le risque de faux positifs.

Afin de déterminer le caractère discriminant d'un biomarqueur, on pourra construire une courbe ROC (Receiving Operator Characteristic) en faisant varier le seuil théorique du biomarqueur testé : on calcule pour chacune des valeurs obtenues dans l'échantillon étudié le couple (sensibilité, 1-spécificité) et on construit le point aux coordonnées correspondantes. La discrimination du biomarqueur sera estimée par l'aire sous cette courbe ROC (AUCROC). Le biomarqueur parfait ayant une sensibilité et une spécificité de 100% aura ainsi une AUCROC à 1, alors que le hasard total aura une AUCROC à 0.5. Ainsi, on considèrera par convention qu'une AUCROC > 0,75 correspond à une bonne discrimination, et qu'au dessus de 0,90 elle est excellente. La construction d'une courbe ROC présente un autre avantage potentiel : déterminer un seuil optimal du biomarqueur. Pour ce faire, plusieurs méthodes mathématiques ou géométriques sont possibles, comme la détermination l'indice de Youden (= Se+Spe-1) qui serait maximum pour le seuil optimal [6].

IV) Interprétation et classification

Un défi majeur du clinicien aux urgences réside dans sa capacité à faire le bon diagnostic, ou d'exclure celui qui devrait l'être. Trivialement, certaines pathologies aiguës nécessitent un traitement rapide pour diminuer la morbi-mortalité [7–11], et à l'inverse, ne pas exclure à tort certaines affections sévères peut augmenter le risque iatrogénique [12–14]. Ainsi, on attend d'un test diagnostique qu'il ait de bonnes performances pour confirmer un diagnostic suspecté (« rule in ») ou pour exclure celui qu'on souhaite écarter (« rule out »). Selon le cas de figure, différentes caractéristiques sont recherchés pour un test diagnostique, et en particulier, différents seuil sont considérés.

En réalité, et l'analyse d'une courbe ROC l'illustre bien, l'amélioration de la sensibilité se fait toujours aux dépends de la spécificité, et vice versa. Ainsi, théoriquement, un test diagnostique a deux seuils : l'un pour exclure le diagnostic (a), et l'autre pour le confirmer (b) comme sur la figure ci-dessous :

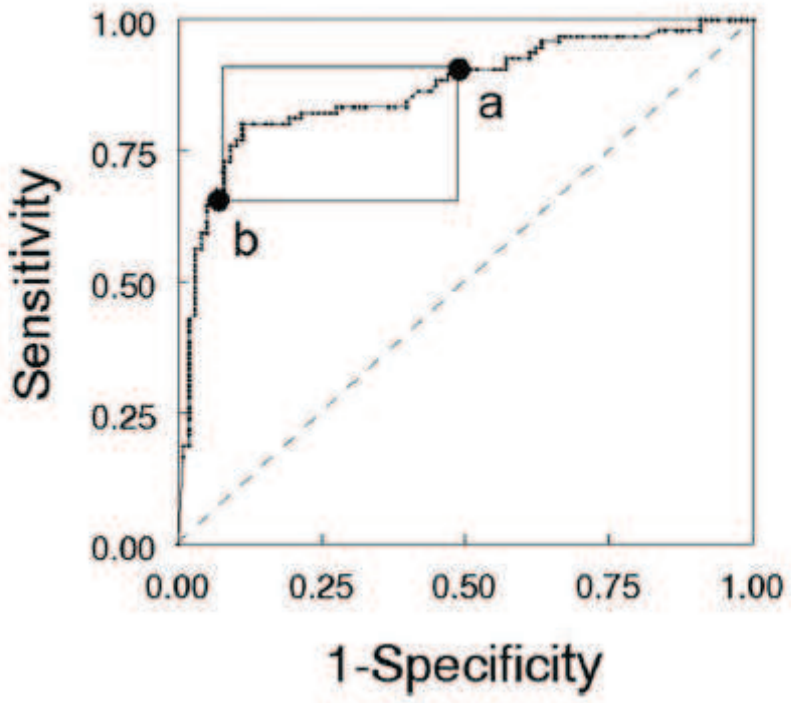

Figure 3 : Courbe ROC du BNP dans le diagnostic d'insuffisance cardiaque aigu, extrait de Ray et al.[6] La zone grise est délimitée entre les point a et b.

En pratique on assiste à plusieurs cas de figures : soit ces deux points sont quasiment confondus et un seul seuil est employé, soit ils sont disjoints et deux seuils sont utilisés, soit enfin, une seule caractéristique importe (confirmer ou exclure) et seul un seuil est conservé.

Par exemple, dans la suspicion de SCA, devant le nombre important de consultation pour des symptômes évocateurs, il faut un test diagnostique qui permette rapidement de faire le diagnostic. Aussi, on prendra comme seuil celui qui donne la meilleure sensibilité – donc la meilleure VPN - mais avec une spécificité très importante. Ainsi, un seul seuil est adopté pour la troponine (cf. développement ci-dessous) qui permet théoriquement l'exclusion ou la confirmation du diagnostic de SCA.

Pour le diagnostic d'insuffisance cardiaque à l'aide du dosage du BNP, la figure ci-dessus illustre bien la « zone grise », et deux seuils sont habituellement choisis : en dessous de l'un, le diagnostic est exclu, au dessus de l'autre, il est confirmé. Entre les deux, le dosage du marqueur n'apporte pas d'information.

Enfin, il n'existe pas de biomarqueur avec une grande spécificité pour le diagnostic d'embolie pulmonaire. En revanche, le dosage de D-dimères a une excellente sensibilité et VPN. Il n'est donc utilisé que pour exclure ce diagnostic - il n'y a pas de seuil au delà duquel la positivité des D-dimères serait capable de le confirmer.

Le résultat d'un biomarqueur peut permettre donc de classer les patients selon leur pathologie par exemple dans le cadre d'un test diagnostique, ou encore classer selon la sévérité pour une évaluation du risque. Ce biomarqueur possède donc des capacités intrinsèques de stratification qui peuvent être évaluées et comparées. On peut par exemple cherche à connaître la valeur ajouté d'un biomarqueur comme test diagnostique en plus de la démarche clinique pour établir le diagnostic d'une pathologie : la clinique classera les patients comme malade ou sain, le biomarqueur aussi, et on peut alors comparer ces deux approches.

Il existe plusieurs méthodes permettant d'évaluer l'amélioration de la stratification, et la reclassification. On citera ici le Net Reclassification Improvement (NRI) et l'Integrated Discrimination Improvement (IDI). On cherche par ces méthodes à estimer le degré de reclassification de patients initialement mal classés.

Introduit par Pencina et al.[15] le NRI représente le gain en certitude du premier test moins celui du second, qu'on peut aussi écrire

$$NRI = (Sensibilité + Spécificité)_{Test} – (Sensibilité + Spécificité)_{test\ de\ référence}$$

Ci-dessous, on montre un exemple d'une matrice de classification pour le diagnostic de SCA par la troponine conventionnelle (référence) et la troponine Hypersensible (dont on veut connaître le gain en reclassification) :

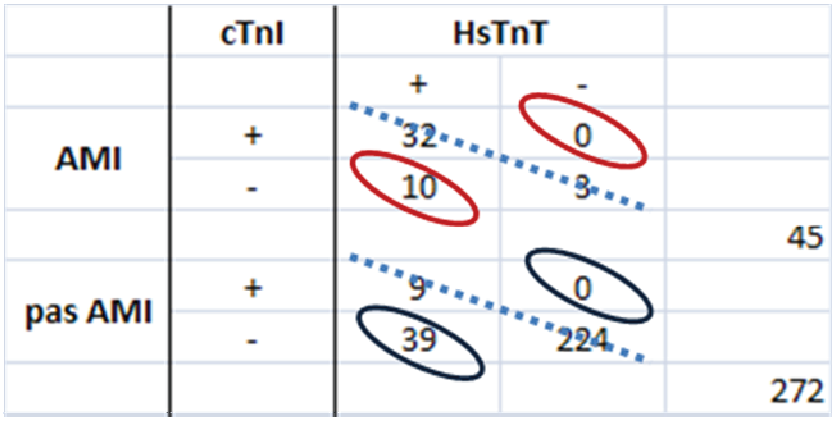

Figure 4 : Tableau de contingence extrait de Freund et al. [16]
En rouge : mieux classés, en bleu : moins bien classés
AMI : Acute Myocardial Infarction (SCA), cTnI : Troponine I Conventionelle
HsTnT : Troponine Hypersensible

On voit qu'il y a (10+0) patients sur 45 mieux classés par la HsTnT parmi les patients avec un diagnostic de SCA, et (39+0) patients sur 272 moins bien classés parmi ceux sans SCA. Le NRI ainsi calculé est à 22%-14% soit 8% avec un Intervalle de confiance à 95% [0.5 – 22]. Cet exemple illustre le cas où le nouveau test améliore la classification. L'exemple ici est donné pour un NRI à deux « classes de risques » (i.e. malade/sain), mais son calcul est possible pour plus de classes, ou encore avec une mesure du risque continue. De même, l'IDI est un indice continu qui prend en compte MID: 17986200","shortTitle":"A multimarker risk stratification approachre le risque par le modèle de prédiction clinique avec et sans le biomarqueur testé, chez des patients avec ou sans le diagnostic recherché.

Ces techniques prometteuses sont actuellement peu rapportées dans les grands essais sur les biomarqueurs, et leur significativité clinique n'est pas évidente. En effet, la capacité de reclassification intrinsèque en soi n'est pas forcément parlante selon le test étudié : les mouvements d'une classe vers l'autre n'ont pas tous la même importance. Par exemple, un patient passant de la classe sain à la classe malade aura probablement moins de conséquences que l'inverse. Une des limites du NRI réside dans cette notion d'égalité entre les mouvements de classes [17]. Par ailleurs, les résultats donnés par ces techniques de NRI ou IDI sont remises en causes au niveau théorique. En particulier, ils seraient moins fiables si le modèle est mal calibré [18,19], et des auteurs rapportent des simulations de modèles avec de bons resultats de NRI et IDI, mais sans aucune valeur ajouté réelle [20].

V) Application : des études à la pratique clinique

On rappelle bien que les résultats d'une seule étude ne sauraient sceller le sort d'un biomarqueur, car ses caractéristiques sont toutes extrapolées d'un seul échantillon, avec ses biais, limites et incertitudes. La validité interne peut être assurée sur une étude par des techniques de ré-échantillonnage (bootstrap) [6], qui consiste a créer un nombre important de nouveaux échantillons constitués de sujets aléatoirement sélectionnés dans la population initiale. Les résultats sur tous ces échantillons nouvellement créés donneront une estimation de l'erreur et de l'intervalle de confiance des résultats initiaux.

Une méthode alternative de validation interne est souvent utilisée, et consiste à diviser une cohorte en deux : l'une pour la dérivation l'autre pour la validation. Cette dernière est cependant très critiquée et peut n'être pas considérée comme valide. La validité externe en revanche passe nécessairement par la réalisation de plusieurs études, sur plusieurs cohortes différentes.

Malheureusement il est fréquent que des résultats encourageant ne soient pas répétés et validés de manière externe. De même, il est fréquent que la validation externe d'une étude soit réalisée certes dans une autre population, mais par la même équipe de chercheur, avec ses même biais et limites [21]. Cette limite est encore amplifiée par le fait que des résultats positifs sont toujours privilégiés à la publication par rapport aux résultats négatifs. Ainsi, un groupe extérieur de chercheurs avec des résultats contradictoire (et négatifs) aurait du mal à mettre en balance les premiers résultats encourageant d'un test diagnostique[*]. Et même dans l'hypothèse d'une confirmation des résultats, le système actuel de publication et de promotion académique ne privilégie pas les recherches originales qui cherchent à vérifier un résultat déjà publié, mais plutôt les études novatrices [21]. Ainsi, dans le domaine des recherches en génétiques, et malgré une accessibilité totale aux données et protocoles des études publiés dans des revues prestigieuses (comme Nature genetics), seules 2 expériences sur les 18 évaluées avaient été reproduites par une autre équipe de chercheurs [22].

[*]*Sans chiffres précis ni preuves réelles, il est admis que les études avec résultats négatifs sont plus difficiles à faire publier que d'autres* [23,24].

La reproductibilité de la recherche et la validation externe des études diagnostiques devraient être des pré-requis avant d'adopter un biomarqueur en pratique clinique. De même que la tenue d'études d'impact. Comme nous l'avons dit précédemment, une étude interventionnelle comparant la stratégie diagnostique habituelle sans et avec le nouveau biomarqueur testé est indispensable pour confirmer l'intérêt de l'adoption d'un biomarqueur en pratique clinique. Ainsi, après avoir démontré les bonnes performances diagnostiques d'un marqueur, son intérêt clinique doit être rapporté. On citera ici l'exemple de la procalcitonine (PCT) qui sera détaillé plus bas : après avoir montré ses excellentes capacités de diagnostic des états septiques aux urgences, l'équipe de P Schuetz et B Mueller ont montré que l'adoption d'une stratégie basée sur le résultat de la PCT permettait une meilleure prise en charge des malades, avec en particulier une réduction de l'exposition aux antibiotiques et leurs effets indésirables, sans aggravation de la morbi-mortalité [25,26].

Assez naturellement, démontrer que l'adoption du biomarqueur en pratique courante est sans risque et avantageuse reste la meilleure façon de prouver qu'il faut adopter ce biomarqueur en pratique courante.

VI) Les biomarqueurs en médecine d'urgence

A la fin des années 1980, les biomarqueurs diagnostiques aux urgences ont gagné leurs lettres de noblesse dans le domaine des pathologies cardiovasculaires. Dès 1978, on retrouve les premières études pilotes évaluant des stratégies pour le diagnostic du SCA basées sur la mesure de protéines musculaires [27]. Au début des années 1980, la myoglobine est étudiée dans plusieurs études [27–30] et fait preuve d'une bonne spécificité pour le diagnostic de SCA. Il en est de même pour la Creatine-Kinase (CK) et la CK-MB [28,31–34]. Mais dès 1990, c'est la troponine qui va s'imposer comme le marqueur doté des meilleures performances diagnostiques dans le SCA [35–40]. L'intérêt et la fiabilité du dosage de troponine dans le diagnostic de SCA est telle qu'elle devient un critère majeur de la définition même du SCA [41]. On retrouve une histoire comparable pour le diagnostic de maladie thrombo-embolique et le dosage de D-dimères : marqueur prometteur testé dans les années 1990 sur de petits échantillons [42,43], il devient marqueur clé pour éliminer ce diagnostic aux urgences dans les années 2000 [44,45].

Ces deux marqueurs ont un commun une grande fiabilité pour leur utilisation (que ce soit la valeur prédictive négative dans la maladie thrombo-embolique, ou la sensibilité pour le diagnostic de SCA), une grande rapidité et précision dans leur mesure, un coût raisonnable, et surtout un impact réel sur la prise en charge des malades aux urgences. L'attrait potentiel que représentent de tels marqueur aux urgences est tel que des dizaines de biomarqueurs déferlent à présent, et qu'il ne s'écoule plus un mois sans qu'un article scientifique évaluant un biomarqueur dans une pathologie ne

soit publié. Pour être adopté en pratique courante aux urgences, un biomarqueur se doit de respecter un cahier des charges comprenant les qualités sus-citées (rapidité/précision de mesure analytique, cout raisonnable, performance statistique au moins comparable à la méthode de référence) ainsi qu'une solide base scientifique qui passe nécessairement par des études d'impact, qui prouverait qu'*in fine*, l'utilisation d'un nouveau biomarqueur possède un réel intérêt concret médical ou socio-économique. Ainsi, depuis 15 ans, seuls deux biomarqueurs diagnostiques ont réussi à s'imposer aux urgences et sont fréquemment utilisés en pratique courante : la procalcitonine (PCT) pour le diagnostic d'infection bactérienne, et le Brain Natriuretic Peptide (BNP) pour celui d'insuffisance cardiaque aigue. Ces deux marqueurs ont en commun une rapidité et une fiabilité de mesure importante, et des études d'impact ont prouvé l'intérêt de leur adoption aux urgences : l'utilisation de la PCT diminue l'exposition aux antibiotiques des patients suspect d'infection respiratoire basse aux urgences [25,26], et l'utilisation du BNP améliore la prise en charge des patients suspect d'insuffisance cardiaque aigue et en diminuerait le coût [46].

Comme nous l'avons décrit précédemment, un biomarqueur peut s'avérer très utile pour estimer la sévérité d'une maladie et les risques de complications, permettant ainsi un traitement et un tri optimisé aux urgences. Sur ce sujet, le biomarqueur clé de gravité est le lactate. Dès 1964, Broder et Weil montrait que l'élévation du lactate était un marqueur important de l'irréversibilité de l'état de choc. De nombreux travaux ont ensuite confirmé l'intérêt pronostique de la valeur du lactate, en particulier dans

les état septiques [47–49]. Ainsi, l'hyperlactatémie est intégrée à la définition même des états septiques sévères [50].

DEUXIEME PARTIE :

Association de biomarqueurs

I) Limite d'un biomarqueur et hypothèse de base

Un biomarqueur aussi bon soit-il, ne peut être parfaitement discriminant. Aussi, mathématiquement, en voulant accroître la sensibilité au-delà d'un certain niveau, la spécificité décroit, et vice versa. Prenons pour exemple le biomarqueur historique clé de la médecine d'urgence : la troponine. Les performances diagnostiques des troponines dite « ancienne génération » étaient excellentes, avec une spécificité de 97% pour le diagnostic de SCA, mais une sensibilité à 71%. Le SCA étant une pathologie aigue avec une mortalité non négligeable, nécessitant un diagnostic et une prise en charge rapide, la sensibilité était insuffisante. Ainsi, de nouveaux tests ont été développés, et plusieurs troponines de nouvelles générations, dites « hypersensibles » ou « ultrasensibles » ont été évaluées. Elles présentent l'avantage d'un seuil de détection avec une précision suffisante significativement abaissé, pour une plus grande sensibilité. Ainsi, pour un seuil de 14 ng/L, la troponine hypersensible présente une excellente sensibilité (93% [16]) pour le diagnostic de SCA, mais avec comme corolaire une spécificité moindre (82% dans notre étude). L'abaissement supplémentaire du seuil aurait comme conséquence de même une diminution de la spécificité, et donc de la valeur prédictive positive. Ainsi, Bandstein et al. ont étudié les performances de la troponine hypersensible lorsque le seuil était abaissé à 5 ng/l : la sensibilité était alors de 98%, mais près d'un patient sur deux présentait un résultat positif, et la valeur prédictive positive était de 12% - rendant difficilement interprétable un résultat positif. On voit bien ici les limites d'une approche basée sur un marqueur.

Un marqueur spécifique de pathologie peut donc présenter d'excellentes caractéristiques diagnostiques en termes de spécificité et de valeur prédictive positive (comme on vient de le voir avec la troponine conventionnelle) : un résultat positif étant très prédictif d'une pathologie. Inversement, un marqueur très sensible présentera d'excellentes caractéristiques au niveau de sa sensibilité et de sa valeur prédictive négative (comme la troponine hypersensible).

Notre hypothèse est que l'association de deux biomarqueurs, un sensible, généraliste et un deuxième spécifique d'organe, pourrait améliorer les performances diagnostiques aux urgences.

II) Principe et histoire de l'approche multimarqueur aux urgences

L'utilisation des biomarqueurs a considérablement aidé les médecins urgentistes à diagnostiquer, stratifier selon la sévérité et à traiter certaines pathologies aiguës comme le SCA, les états septiques graves, l'embolie pulmonaire ou encore l'insuffisance cardiaque aiguë. De nombreux signes cliniques, scores, ou marqueurs biologiques existent pour guider le clinicien dans sa pratique, avec une valeur ajoutée plus ou moins probante, mais aucun de ces marqueurs ne peut être considéré comme parfait et se suffire à lui-même dans sa fonction, qu'elle soit diagnostique ou pronostique.

Certains biomarqueurs ont donc été évalué et utilisé en association avec un score clinique par exemple, pour améliorer leurs performances diagnostiques (comme par

exemple les D-dimères en association avec le score de Genève pour les suspicions d'embolie pulmonaire).

De même, est apparu récemment l'idée d'utiliser une combinaison de différents biomarqueurs pour améliorer leurs performances. Les premiers travaux dans ce sens s'intéressaient au diagnostic de SCA. Comme on l'a vu précédemment, plusieurs biomarqueurs diagnostiques du SCA ont été évalués dans les années 1980 (CK, CK-MB, Myoglobine) puis dans les années 1990 (Troponine T, Troponine I). Naturellement, plusieurs études diagnostiques ont cherché à comparer les performances de ces différents marqueurs. Les sensibilités, spécificités, valeurs prédictives négatives et positives de ces marqueurs sont donc comparés sur des populations différentes selon les études, afin de déterminer quel est le marqueur le plus utile au clinicien pour le diagnostic de SCA aux urgences. Dès 1983, Grenadier et al. rapportent que la myoglobine est plus sensible que les CK et CK-MB pour le diagnostic d'infarctus du myocarde [51], ce qui a été confirmé plus tard par plusieurs études (on citera Bakker et al. [52], Kilpatrick et al. [53], Brogan et al.[54] ou encore Zabel et al qui s'intéressaient à la cinétique de ces marqueurs) [55]. Ces travaux ont été suivis de celui d'Apple et al. qui jette les bases de la supériorité de la troponine par rapport à ces biomarqueurs [56].

Mais dès 1995, plutôt que d'évaluer séparément ces biomarqueurs d'intérêt, plusieurs équipes ont cherché à évaluer leur apport combiné. Ainsi, Thomson et al. rapportent sur une étude de 511 patients aux urgences que la combinaison de l'élévation des CK-MB et de la présence d'une lymphocytopénie induite par le cortisol avait une spécificité presque parfaite et une VPN très élevée (respectivement 99% et

94%) [57]. Puis l'année suivante, Levitt et al. comparaient les caractéristiques de la myoglobine et des CK-MB, seuls, et en association [58] : la combinaison des deux (i.e. positivité de l'un ou de l'autre) avait une sensibilité et une spécificité supérieures à celles de ces marqueurs seuls – résultats similaires rapportés peu après par Kontos et al.[59]. La troponine a supplanté peu à peu ces autres marqueurs à la fin des années 1990 et a été tout naturellement testé aussi en association, comme dans l'étude de Sabatine et al. [60] en 2002, qui trouvait qu'une approche multimarqueurs incluant la troponine, la C Reactive Protéine (CRP) et le BNP était plus performante pour la prédiction d'un critère composite de SCA, oedème aigü du poumon (OAP) ou décès lorsque les trois marqueurs étaient pris en compte, plutôt que chacun séparément. Ces résultats ont été confirmés 5 ans plus tard avec l'étude de Tello-Montoliu et al. [61].

En dehors de la douleur thoracique, l'approche multimarqueurs a de même montré son intérêt potentiel dans d'autres contextes aux urgences. Ainsi, en 2009 Shapiro et al. ont présenté une première étude évaluant une combinaison de biomarqueurs pour le diagnostic et le pronostic des états septiques sévères : la combinaison du dosage de la Neutrophil Gelatinase-associated Lipocalin (NGAL), de l'Interleukine-1ra, et de la CRP était prédictive d'état septique sévère et de décès avec une AUCROC autour de 0,8 [62]. Chez les patients présentant une dyspnée aiguë, de même, Christ et al. rapportent un risque accru de décès ou d'hospitalisation selon le nombre de biomarqueurs qui s'élèvent, parmi BNP, troponine, et CRP [63].

Ces premiers résultats encourageants mais loin d'être définitifs ouvraient la voie à l'évaluation d'association de biomarqueurs à la recherche d'un modèle aux performances optimales.

III) Analyse statistique d'une approche multimarqueurs

Nous allons ici reprendre les bases de l'analyse statistique d'une combinaison de biomarqueurs.

La méthode la plus classique permet une vraie interprétation clinique, et est facilement utilisable en pratique par le clinicien. Il s'agit de celle utilisée dans les études que nous venons de citer. On reprend l'exemple du SCA : les biomarqueurs sont évalués séparément par leurs caractéristiques diagnostiques de base (sensibilité, spécificité, VPP et VPN). Elle provient d'un réel besoin clinique avec application immédiate : devant un patient suspect, quelle information m'indique la valeur de mon biomarqueur ?

Ainsi, la troponine ultrasensible a été rapidement adopté car sa valeur prédictive négative quasi parfaite en fait un atout de choix dans l'évaluation des patients suspects de SCA : si la valeur de la troponine ultrasensible d'un patient avec une douleur thoracique depuis plus de 4 heures est inférieur à la norme, ce diagnostic peut être exclue. L'approche multimarqueur sur le SCA dans les années 1990 se basait sur ce principe simple : on considérait l'association de biomarqueur comme un nouveau marqueur et on calculait ses caractéristiques diagnostiques. Par exemple,

dans l'étude de Levitt et al. [58], les auteurs évaluent les CK-MB et la myoglobine séparément, ainsi que leur combinaison dont la positivité est définie par « l'un ou l'autre des deux marqueurs est positifs ». Les seuils de chacun étaient déterminés par la construction de la courbe ROC, et le nouveau marqueur combinaison pouvait alors être évalué – non comme une variable continue mais comme une variable binaire. Une fois cette variable binaire obtenue, les principes statistiques de bases développés plus haut s'appliquent, et on peut calculer sensibilité, spécificité, VPN, VPP, RV+, et RV- de toute combinaison de marqueurs dont on connaît les seuils. On peut procéder de même en définissant la positivité de la combinaison comme étant la combinaison de l'un ET de l'autre des biomarqueurs. Cette approche permet d'améliorer la spécificité, au détriment de la sensibilité. Les qualités de reclassifications peuvent de même être évaluées grâce à ces combinaisons pour en faire une variable binaire, et les techniques de NRI ou IDI sont utilisables.

Selon l'utilisation clinique qui est attendue, on choisira l'une (« ET ») ou l'autre (« OU ») des combinaisons. En reprenant l'exemple de la suspicion de SCA aux urgences, le clinicien peut souhaiter favoriser la sensibilité et la valeur prédictive négative, et donc chercher des combinaisons de biomarqueurs sous forme de positivité de l'un OU de l'autre.

Au-delà de deux marqueurs, il est toujours possible de construire de nouveaux marqueurs comme combinaison de l'un ET l'autre, ou l'un OU l'autre, mais le nombre de combinaisons possibles augmente exponentiellement : en effet, il y a 2^{n-1} possibilités d'associer n biomarqueurs.

Dans ces cas de figures, il est possible d'utiliser un score, selon le nombre de marqueurs positifs. On peut alors évaluer cette variable, représentant l'association de biomarqueur, et en déduire d'éventuelles propriétés diagnostiques ou de stratification du risque. Par exemple, on peut étudier le risque d'être atteint d'une pathologie selon le nombre de marqueurs positifs, ou encore la survie selon la valeur de ce score.

Ces combinaisons sont appelées les « combinaisons logiques » - combinaisons de « ET » et « OU »- et sont préférées par les cliniciens pour des raisons de simplicité et d'interprétabilité [64].

Enfin, il est possible d'aborder l'approche multimarqueurs comme une variable quantitative, combinaison de plusieurs variables continues. Une combinaison linéaire par exemple peut être étudiée. Si cette combinaison est une variable quantitative, il est alors possible de construire sa courbe ROC et de calculer son AUC_{ROC}. Il convient alors de déterminer la combinaison linéaire qui maximise cette aire sous la courbe, et donc le pouvoir discriminant de la combinaison. On appelle cette combinaison linéaire la BLC (Best Linear Combination). Plusieurs méthodes peuvent être utilisées pour déterminer les coefficients optimaux d'une BLC Ω

$$\Omega = aA+bB+cC\ldots$$

Su et Liu en 1993 proposaient une formule dérivée de la matrice de covariance des différents marqueurs pour construire la BLC, combinaison qui maximise l'AUC_{ROC} [65–67]. Les auteurs ont ainsi démontré que sous certaines conditions (normalité de la distribution des biomarqueurs étudiés, dans la population saine et dans la population

malade), les coefficients pouvaient se déterminer comme un vecteur U (a1, a2, a3, …)'

Qu'on peut calculer comme étant le produit matriciel suivant :

$$(\Sigma^T + \Sigma^M)^{-1}(\mu^M - \mu^T)$$

Σ étant la matrice de covariance des différents biomarqueurs dans les populations malades (Σ^M) et saines (Σ^T), et μ le vecteur dont les coordonnées sont les moyennes de chaque biomarqueur dans les populations malades et saines. Derrière cette formule (dont la démonstration complète est retranscrite dans l'article princeps [65]) on voit bien qu'un poids plus important est attribué aux biomarqueur plus discriminants et avec des variances plus petite. Le deuxième produit par exemple est directement la différence des moyennes du marqueur entre population saine et malade.

Pour plus de clarté, on applique en exemple cette formule au cas simple de la combinaison Ω = aA+bB de deux biomarqueurs A et B dont la distribution est normale dans les populations saines $\begin{pmatrix} A \\ B \end{pmatrix}$ = X~(μ_x, Σ_x)) et malade $\begin{pmatrix} A \\ B \end{pmatrix}$ = Y~N(μ_y, Σ_x).

La formule de Su et Liu donne

$$U = (a,b)' = (\Sigma_x + \Sigma_y)^{-1}(\mu_x - \mu_y)$$

Les matrices de covariances dans chaque population étant :

$\sum = \begin{matrix} \sigma^2 A & \sigma AB \\ \sigma AB & \sigma^2 B \end{matrix}$, où σ² est la variance de chaque biomarqueur, et σ_{AB} la covariance de A et B – respectivement dans les populations saines ($\Sigma_{x)}$ et malades ($\Sigma_{x)}$. On peut

ensuite faire l'hypothèse de proportionnalité entre les deux matrices de covariances [65] *(ceci simplifie la présentation mais le calcul reste tout aussi aisé, sans cette hypothèse)* soit $\mathbf{\Sigma_x = k\Sigma_y}$. Ainsi on obtient :

$$\begin{pmatrix} a \\ b \end{pmatrix} = \kappa \begin{pmatrix} \sigma^2{}_A & \sigma_{AB} \\ \sigma_{AB} & \sigma^2{}_B \end{pmatrix}^{-1} \begin{pmatrix} \hat{\mu}_A \\ \hat{\mu}_B \end{pmatrix}$$

ou $\hat{\mu}A$ est la différence des moyennes de A entre populations malades et saines *(Id pour B)*. L'inversion de la matrice fait intervenir son déterminant, et à un coefficient κ' près on obtient :

$$\begin{pmatrix} a \\ b \end{pmatrix} = \kappa' \begin{pmatrix} \sigma^2{}_B & -\sigma_{AB} \\ -\sigma_{AB} & \sigma^2{}_A \end{pmatrix} \begin{pmatrix} \hat{\mu}_A \\ \hat{\mu}_B \end{pmatrix}$$

La courbe ROC étant indifférente à un coefficient multiplicateur près, le problème de la maximisation de son AUC pour deux biomarqueurs revient donc à déterminer le coefficient α tel que C=A+αB ait une AUCROC maximale. Ce coefficient est donc donné par :

$$\alpha = b / a = \frac{\sigma^2{}_A . \hat{\mu}_B - \sigma_{AB} . \hat{\mu}_A}{\sigma^2{}_B . \hat{\mu}_A - \sigma_{AB} . \hat{\mu}_B}$$

Une alternative consiste à attribuer comme coefficients les Odds Ratios issues d'une régression logistique binaire, identifiant les différents prédicteurs indépendants [68]. Cette méthode, plus simple à mettre en œuvre, n'apporte cependant aucune garantie quand à l'optimisation de la combinaison.

IV) Biomarqueurs spécifiques d'intérêt potentiel aux urgences :

A. La procalcitonine

La procalcitonine (PCT) est une pro-hormone, précurseur de la calcitonine, et est composée de 116 acides aminés. Après élimination d'une séquence de 25 acides aminés, le premier produit de la calcitonine (la pre-procalcitonine) devient la procalcitonine. La concentration de PCT à l'état physiologique est très faible (<0.1 µg/l). En revanche, lors d'états septiques, les concentrations peuvent être très augmentés. C'est depuis 1993 en pédiatrie que ce marqueur sérique a révélé son potentiel dans le diagnostic des méningites. Sa spécificité aux infections bactériennes, contrairement à la CRP, peut s'avérer d'un grand intérêt aux urgences. En prenant comme seuil 0,5 µg/l, la spécificité rapportée est proche de 99% [69,70] ce qui peut permettre une identification rapide des infections bactériennes, et la mise en route d'un traitement antimicrobien adapté. Les qualités diagnostiques de la PCT ont été souvent démontrées et publiées, en particulier pour le diagnostic d'infection respiratoire basse. Les études d'impact ProHOSP et ProREAL ont montré que l'adoption de ce biomarqueur en pratique clinique courante améliorait la prise en charge des patients aux urgences avec suspicion d'infection respiratoire basse [25,26].

De plus, la concentration de PCT semble être corrélée à la gravité de l'infection bactérienne, et pourrait être prédictif d'état septique sévère (sepsis sévère ou choc septique) [69,71,72]. L'échantillon de choix pour le dosage de la PCT est le sérum, mais il est possible de mesurer la PCT dans le plasma.

B. La protéine S100 Béta

La protéine S100-Beta (S100B) appartient à la famille des protéines de liaison du calcium intracytosolique. Elle est constituée de deux parties (soit deux parties « béta », soit une « alpha » et une « béta »). Sa neurospécificité provient de la sous-unité Béta, essentiellement synthétisée par les cellules astrogliales. On la retrouve aussi très faiblement exprimée par les mélanocytes, adipocytes et chondrocytes. Son dosage se réalise habituellement sur sérum, voire sur plasma. Dans une population saine, sa concentration est généralement inférieure à 0,1 µg/l.

Son intérêt a été initialement décrit en post arrêt cardiaque, pour estimer le pronostic neurologique. Ainsi, un taux élevé de S100B était prédictif d'un mauvais pronostic neurologique [73,74]. Au cours de la dernière décennie, de nombreuses études ont rapporté son intérêt dans le pronostic neurologique après un accident vasculaire cérébral (AVC) [75,76]. Récemment aux urgences, l'intérêt de la S100B s'est précisé du fait de son potentiel dans l'évaluation précoce du risque de complication neurologique après un traumatisme crânien. En 2006, Biberthaler et al. ont montré que l'utilisation de règles décisionnelles basées sur la valeur de la S100B pouvait diminuer de 30% le nombre de scanner cérébraux prescrit après traumatisme crânien mineur. Ces résultats ont été corroborés par la suite, en particulier avec les études françaises de Zongo et al. et Laribi et al. : la valeur prédictive négative de la S100B mesurée après traumatisme crânien mineur pour l'exclusion de lésion cérébrales était supérieure à 99.5% [77,78]. Ainsi, l'utilisation en pratique courante de la S100B permettrait de sécuriser la prise en charge des patients, et de diminuer le nombre d'imagerie cérébrale aux urgences.

C. La troponine

La troponine a révolutionné la prise en charge de l'infarctus du myocarde depuis les années 1990, à tel point que sa valeur (ou sa variation) entre dans la définition même de l'infarctus du myocarde [41]. Une valeur supérieure au 99ème percentile d'une population saine est pathologique et considérée comme une souffrance myocardique. La précision de la mesure d'un biomarqueur peut être exprimée par son coefficient de variation (CV). Ce CV, pour une valeur donnée, rend compte de l'imprécision de la mesure. Il est accepté que ce CV ne doive pas dépasser 10% pour que le résultat soit précis. Ainsi, il existe un seuil en dessous duquel la mesure d'un biomarqueur peut ne plus être fiable – la limite de quantification (LoQ). Les troponines dites d'anciennes générations (par exemple la cTnI de Siemens ®) présentaient un CV supérieur à 10% au 99ème percentile, ce qui empêche l'utilisation de ce seuil. En effet, la LoQ était à 0.14 µg/l, et le 99ème percentile à 0.07 µg/l. Ainsi, les troponines d'anciennes générations ne pouvaient permettre le diagnostic de syndrome coronaire aigue pour des valeurs pathologiques mais faibles de troponines. L'apparition de troponines « hyper sensibles » ou « ultra sensibles » a permis de corriger ce défaut, grâce à une LoQ inférieure au 99ème percentile. Ces nouvelles troponines permettent donc de détecter avec une précision < 10% des concentrations proches du 99ème percentile.

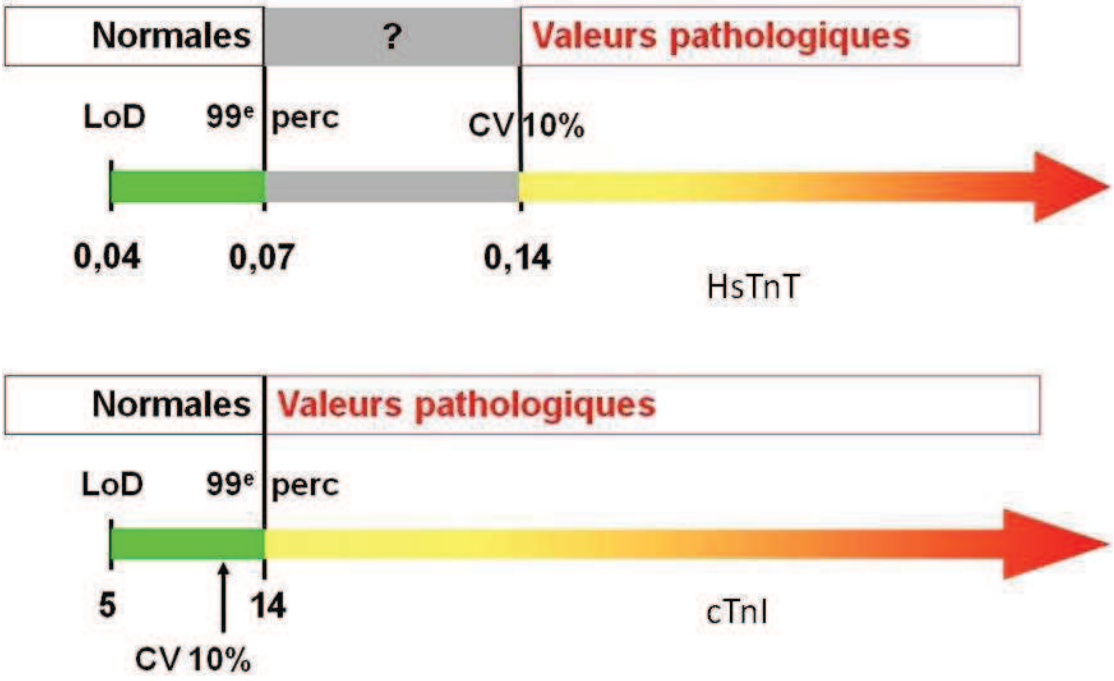

Figure 5 : Reproduit avec l'aimable permission du Dr C. Chenevier-Gobeaux CV : coefficient de variation, LoD : limite de détection. HsTnT : troponine hypersensible. cTnI : troponine conventionelle.

V) Biomarqueurs généralistes d'intérêt potentiel

A. La copeptine

La copeptine, tout comme la vasopressine, provient de la pré-vasopressine qui est synthétisée dans l'hypothalamus. La copeptine est libérée en réponse à un stress physiologique intense. Plus stable et de mesure plus fiable, sa valeur reflète la concentration de vasopressine, et permet cette évaluation en pratique courante [79]. Les valeurs normales de la copeptine sont habituellement inférieures 14 pmol/l [79]. Une élévation de la copeptine traduirait donc un stress physiologique, et la rapidité de son élévation en fait un marqueur d'intérêt notable pour une utilisation aux urgences. Dès 2007, son intérêt pronostique à court et long terme a été décrit par

Stolz et al. dans le cadre des décompensations aiguës de BPCO [80]. Des travaux ont de même relevé son intérêt dans le pronostic et la stratification de la sévérité des dyspnées aiguës [81,82]. Mais c'est surtout pour le diagnostic de SCA que la copeptine a montré tout son potentiel : elle permettrait d'exclure ce diagnostic dès les premières heures de présentation, alors que la troponine, marqueur référence, nécessite quelques heures avant son élévation. Ainsi, en 2009, Reichlin et al. ont relevé l'apport de la copeptine dans l'exclusion du SCA, avec une valeur prédictive négative supérieure à 99,5%. Enfin, récemment, l'étude interventionnelle BIC-8 évaluait une stratégie d'exclusion précoce du diagnostic et une sortie des urgences en cas de résultat négatif pour la troponine et la copeptine. Cette stratégie n'augmentait pas le taux d'évènements indésirables cardio-vasculaires majeurs, et diminuait significativement le taux d'admission ainsi que la durée de séjour.

B. Le lactate

Le lactate est un métabolite issu de l'aboutissement de la glycolyse et s'accumule lorsque le pyruvate ne peut être métabolisé, comme par exemple en cas d'hypoxie tissulaire. Dès 1964, les travaux de Broder et Weil retrouvent une association entre hyperlactatémie et sévérité du choc [83]. La surmortalité des patients en hyperlactatémie a été par la suite maintes fois décrite, dans des situations diverses [49,84,85]. Dès 1992, la publication par Bone et al. de la conférence de consensus de l'American College of Chest Physicians et la Society of Critical Care Medicine (ACCP / SCCM) sur les états septiques a intégré l'hyperlactatémie dans la définition du sepsis sévère [86]. Depuis 20 ans, l'utilité du dosage du lactate aux urgences a été rapportée dans de nombreux domaines. Dans l'évaluation des états septiques dès l'admission par exemple, les travaux de Mikkelsen et al. et Shapiro et al. montrent bien l'intérêt du lactate dans la stratification du risque des états septiques[47,48]. Les actualisations de la conférence de consensus sus-cité et les recommandations de la Surviving Sepsis Campaign ont confirmé le rôle central du dosage du lactate dans l'appréciation de la gravité des états septiques [50,87,88].

L'hyperlactatémie est de même associée à une surmortalité ou plus grande morbidité après arrêt cardiaque [89–91], dans l'embolie pulmonaire [92], ou encore chez le patient traumatisé [93–97]. En pédiatrie aussi, Scott et al. ont rapporté l'intérêt du dosage du lactate chez les enfants présentant un syndrome de réponse inflammatoire systémique (SIRS) : l'hyperlactatémie précoce était fortement associée au risque de dysfonction d'organe [98].

Enfin, plus que la valeur absolue du lactate, la clairance de celui-ci serait un facteur encore plus utile pour l'évaluation des patients aux urgences. Tant dans le sepsis que chez le polytraumatisé, il est bien montré que la diminution du taux de lactate est un facteur prédictif d'amélioration clinique [93,99–103]. Ainsi, c'est peut être moins la valeur initiale du lactate que son évolution immédiate après traitement qui devrait guider le clinicien dans sa prise en charge.

TROISIEME PARTIE :

Etudes cliniques

I) Article 1 : Procalcitonine et Lactate dans le sepsis

A. Introduction

Le sepsis grave a une incidence annuelle de 3 pour 1000 dans la population américaine[104], et jusqu'à 5 pour mille en France (d'après le Groupe Transversal Sepsis). Sa mortalité reste très élevée, malgré une légère décroissance depuis le début des années 2000 [105]. Les patients des urgences représentent une large proportion de cette population, avec une mortalité rapportée entre 20 et 50% [104,106]

Même si les résultats de Rivers et al. sur l' « early goal directed therapy » sont actuellement remis en cause [106,107], il n'en reste pas moins que la reconnaissance précoce d'un état septique sévère et sa prise en charge rapide sont des déterminants forts du pronostic vital de ces malades [7,108]. Aux urgences, la présentation de tels patients n'est pas toujours évidente, et les signes de choc ou de défaillance d'organe ne sont pas forcément francs et visibles dès le début de la prise en charge. L'identification rapide des états septiques sévères est parfois malaisée [7,109], aussi l'utilisation de biomarqueurs pourrait aider le clinicien à améliorer et accélérer la reconnaissance de telles pathologies.

Une élévation du lactate reflète une hypoperfusion tissulaire et est indépendamment associée à une morbi-mortalité plus élevée [47,48], ce qui en a fait une partie intégrante des critères diagnostiques du sepsis sévère [50]. Son dosage dès la première heure de prise en charge est recommandé pour aider le clinicien à identifier rapidement et à traiter de manière appropriée ce syndrome grave. La procalcitonine est un biomarqueur du sepsis assez spécifique de l'infection bactérienne et qui peut être

utilisé pour guider la décision d'antibiothérapie [25,26,110]. De plus, son élévation semble associée à la gravité du sepsis [69,71,72].

L'apport singulier de chacun de ces deux biomarqueurs dans la prise en charge des états septiques aux urgences est bien connu, mais l'intérêt de leur combinaison reste peu étudié. L'hypothèse de cette étude est que ces deux marqueurs ont un intérêt complémentaire.

B. Discussion

Cette étude rétrospective monocentrique suggère que le dosage conjoint de la procalcitonine et du lactate apporte des informations complémentaires. Ainsi, parmi la population présentant une anomalie d'un seul de ces deux marqueurs, moins d'un quart des patients (22% pour la PCT et 24% pour le lactate) auront une évolution défavorable (admission en réanimation ou décès), alors qu'ils seront 56% parmi ceux qui ont à la fois une hyperlactatémie et une procalcitonine élevée. Ces deux dosages ne sont ainsi pas redondants dans le sepsis, mais semblent bien complémentaires. De même, la positivité des deux marqueurs semble plus prédictive d'aggravation secondaire que la positivité de l'un ou l'autre.

Alors que ces deux marqueurs semblent avoir une acuité à peu près équivalente pour prédire une évolution défavorable, leur intérêt semble se potentialiser lorsqu'ils sont tous deux dosés. Si on s'intéresse aux caractéristiques diagnostiques isolées de ces deux marqueurs, on voit bien tout l'intérêt de leur combinaison :

En prenant la combinaison « l'un ou l'autre positif », la sensibilité est significativement améliorée : 72% vs 54% ou 51%. A l'inverse, en cherchant la positivité « l'un ET l'autre », on augmente la spécificité qui est à 93% pour la combinaison vs 76% ou 75%.

Le dosage combiné de la PCT et du lactate semble être un bon outil diagnostique et pronostique dans l'évaluation des états septiques aux urgences.

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RESEARCH ARTICLE

Serum lactate and procalcitonin measurements in emergency room for the diagnosis and risk-stratification of patients with suspected infection

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Abstract

Objective: To study the contribution of lactate and procalcitonin (PCT) serum measurements for the diagnosis and the risk-stratification of patients with suspected infection presenting to the ED.

Methods: Single-center one year observational study on 462 consecutive patients. Multivariate analysis to assess variables associated with sepsis, severe sepsis, septic shock and severe outcome.

Results: Multivariate analysis (Odds ratio [95% CI]), showed that PCT was the best independent variable to identify sepsis (3.98 [2.60–6.10]), while lactate was the best to diagnose severe sepsis (10.88 [6.51–18.19]). Patients with both lactate above 2 mmol.L^{-1} and PCT above 0.8 ng.mL^{-1} had an enhanced risk of severe outcome.

Conclusions: the dosages of lactate and PCT are complementary for the diagnosis and risk-stratification of patients evaluated in the ED for suspected infection.

Keywords: Lactate, procalcitonin, sepsis, septic shock, diagnosis, prognosis, ED

Introduction

The accurate evaluation of patients with suspected infection is a major concern for emergency physicians, since early specific therapeutic management correlates with better outcome (Rivers et al. 2001). However, signs of organ dysfunction or cryptic shock may not be obvious for the physician at the time of patient's presentation. Moreover, the wide clinical polymorphism and the earlier presentation of septic patients at the emergency department (ED) (in comparison to intensive care units, ICU) and the organizational features and constraints (as overcrowding) may contribute to misdiagnosis. Therefore, sepsis biomarkers may be useful in addition to clinical evaluation to improve both the diagnosis and severity assessment of septic patients. High serum lactate level reflects critical tissue hypoperfusion and is associated with increased morbidity and mortality in critically ill patients and particularly in patients with severe sepsis or septic shock (Bakker et al. 1996, Vincent et al. 1983, Nguyen et al. 2004, Jansen et al. 2009). The usefulness of serum lactate measurement, as a severity biomarker, has been well established in intensive care units (ICU) but has only recently been confirmed in the ED (Shapiro et al. 2005, Howell et al. 2007, Mikkelsen et al. 2009). Procalcitonin (PCT) is a sepsis biomarker that exhibits enhanced specificity for the bacterial origin of infection (Assicot et al. 1993, Hausfater et al. 2002, Christ-Crain et al. 2004, Hausfater et al. 2007). Morevorer, PCT levels

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Abbreviations

AUCROC, area under the ROC;
CRP, C reactive protein;
ED, emergency department;
HIV, human immunodeficiency virus;
ICU, intensive care unit;
PCT, procalcitonin;
ROC, receiver operating curve;
SBP, systolic blood pressure;
SD, standard deviation;
SIRS, systemic inflammatory response syndrome;

have been reported to be associated with the severity of infection either in ED or in ICU settings (Ugarte et al. 1999, Hausfater et al. 2002, Hausfater et al. 2007). However, the respective performance of lactate and PCT measurement, as well as the added-value of their concomitant dosages for the evaluation of patients suspected to have sepsis, has not been extensively studied in ED.

The aim of the present study was to determine the respective contribution of lactate and PCT measurement for the diagnosis and the prognosis of patients with suspected infection presenting to the ED. We made the hypothesis that these two biomarkers may provide complementary information that could be useful in a multiple biomarkers approach.

Methods

Patients

This was an observational cohort study of consecutive patients presenting during a 12-month period to the ED of an urban academic 1600 bed hospital with a 55,000 annual admissions to the ED. Patients 15-year-old or greater were included if they presented with a suspected diagnosis of infection to our ED during the study period and had available both lactate and PCT serum measurements blood sampled in the emergency room. PCT and lactate measurements are performed in routine practice in our ED in cases of suspected infection, and both biomarkers results are available in 1 h. All blood samples studied were drawn before any therapeutic intervention. Because of the observational design of the study, the ethical committee (CPP Ile de France Paris VI, Paris, France) authorized a waiver of informed consent. For patients with multiple measurements, only the first blood sample was taken into account. A trained research assistant reviewed each electronic ED file and recorded admission data (including first vital variables measured and routine biological data at entry and diagnosis retained in ED) and outcome (discharge, admission to a medical ward or ICU, secondary transfer into an ICU, in hospital mortality). For each patient, the presence of systemic inflammatory response syndrome (SIRS), sepsis, or severe sepsis/septic shock criteria (Levy et al. 2003) were also systematically recorded, either at ED admission or during follow-up. However, for this study we kept hyperlactatemia as a severe sepsis criterion but did not take into account PCT value for sepsis criteria. As a high lactate level is not specific of severe sepsis (Fall & Szerlip 2005), patient's electronic files were screened for associated factors that may contribute to raised serum lactate levels (cancer, alcoholic consumption, inhaled or systemic β-2 agonists, statin or antiretroviral treatment, diabetes mellitus, anemia, seizures and shock of other origin than sepsis). However, we did not exclude the patients with such associated causes of high lactate levels. We further categorized two outcome subgroups of patients defined as follows: severe outcome (any death and/or ICU admission (either primary or secondary) and/or terminal patients with therapy limitations) and secondary worsening (secondary admission in ICU and unexpected deaths, i.e. deaths that occurred in patients that were initially not considered to require ICU admission, and were not terminal patients with therapy limitations).

Biological measurements

Procalcitonin was measured by a time-resolved amplified cryptate emission (TRACE) technology assay (Kryptor PCT; Brahms, Hennigsdorf, Germany). This assay is based on polyclonal antibody against calcitonin and a monoclonal antibody against katacalcin which bind to calcitonin and katacalcin sequence of precursor molecules. The limit of detection was 0.02 ng.mL^{-1}. Normal values were <0.1 ng.mL^{-1} and the functional sensitivity was 0.06 ng.mL^{-1}.

Lactate was measured by an enzymatic method (lactate-oxidase) in whole arterial blood using a Radiometer ABL 725 blood gas analyzer (Radiometer Medical A/S, Neuilly-Plaisance, France), or in venous plasma-based assays on Roche Cobas Integra 400 plus analyzer (Roche Diagnostics, Meylan, France). The normal range was 0.5–1.8 and 0.5–2.2 mmol.L^{-1} in arterial and venous blood respectively.

Statistical analysis

Data are expressed as mean ± SD or median (25–75 interquartile range) in non-normally distributed variables (Kolmogorov–Smirnov test). Comparisons between two groups were performed using the Student's *t*-test, the Mann–Whitney test, and Fisher's exact method, when appropriate. The Bonferroni correction was applied for multiple comparisons. Comparison of two medians in the same sample was performed using the Wilcoxon test.

We determined the receiver operating curve (ROC) and calculated the area under the ROC curve and its 95% confidence interval. The ROC curve was used to determine the optimal threshold for PCT and lactate to accurately identify the following criteria: sepsis, severe sepsis, septic shock and severe outcome (as defined previously). The optimal threshold was the one which maximizes the Youden index (sensitivity + [specificity – 1]) on the ROC curve (Ray et al. 2010). Comparison of areas under the

ROC curve was performed as previously described by Delong et al. (1988).

We performed a multivariate analysis to assess variables associated with sepsis, severe sepsis, septic shock and severe outcome using backward logistic regression. To avoid overfitting, we used a conservative approach and included only the significant variables in the univariate analysis (p value of entry ≤ 0.10). Interactions were not tested. The odds ratio and their 95% confidence interval of variables selected by the logistic model were calculated. The discrimination of the model was assessed using the ROC curve and the calculation of the area under the ROC curve. The percentage of patients correctly classified by the logistic model was calculated using the best threshold determined by the ROC curve. Calibration of the model was assessed using the Hosmer-Lemeshow statistics.

We also calculated the main diagnostic variables (sensitivity, specificity, negative and positive predictive values, positive and negative likelihood ratios) and their 95% confidence interval associated with a severe outcome when considering elevated PCT, elevated lactate, one of these, or both of them.

All p values were two-tailed and a p value of less than 0.05 was considered significant. Statistical analysis was performed using NCSS 6.0 software (Statistical Solutions Ltd, Cork, Ireland) and R software with specific packages (http://www.R-project.org).

Results

During the 12-months study period, 462 patients suspected of being infected underwent both PCT and lactate serum measurements at admission. There were 272 (59%) men and 190 (41%) women; mean age was 64 ± 20 years (range 15–102 years). The cohort comprised 58 patients with cancer ongoing treatment, 15 HIV-infected patients, 7 patients with multiple sclerosis and 4 with systemic vasculitis ongoing corticosteroid therapy. The main ED admission characteristics of these patients are summarized in Table 1. Samples for lactate measurement were drawn mostly from arterial puncture (82%) and the remaining from peripheral venous. One hundred and forty patients (30%) had a lactate >2 mmol/L and 35 (8%) >4 mmol/L. Two hundred and fifty-six patients (55%) had, at admission 2 or more SIRS criteria, 283 (61%) had sepsis, 117 (25%) severe sepsis and 10 (2%) septic shock.

Overall, there were 86 patients who were initially considered to be critically ill (at least one vital failure) including 12 terminal patients with therapy limitations, 15 patients who secondarily became so (2 unexpected deaths and 13 secondary ICU admissions) and 361 patients who remained definitely noncritically ill. Finally, 87 (19%) patients were admitted to the ICU (74 directly from the ED and 13 in the following days after initial admission on a medical bed) (Figure 1). Overall, 20 patients (4%) died, thus generating a severe outcome subgroup size of 101 (22%) patients (Figure 1). The secondary worsening subgroup comprised 15 patients with

Table 1. Main baseline characteristics of patients at ED admission (n = 462).

Variables	N	n (%) mean ± SD median (25–75 IQR)
Age	462	64 ± 20
Age > 75 years	462	173 (37%)
Sex male		272 (59%)
Baseline characteristics		
Temperature (°C)	462	37.3 ± 1.1
Heart rate (beats per minute bpm)	462	98 ± 23
Systolic blood pressure (mmHg)	459	127 ± 25
Pulse oximetry	457	95 (92–98)
Temperature > 38°C or < 36°C		130 (28%)
Heart rate > 90 bpm		283 (61%)
Systolic blood pressure < 90 mmHg		25 (5%)
Pulse oximetry < 90%		76 (17%)
Biology		
White blood cell count (per mm^3)	458	11 313 ± 7162
Creatinine (μmol.L^{-1})	459	111 ± 113
Lactate (mmol. L^{-1})	462	2.02 ± 1.71
Lactate > 2		140 (30%)
Lactate > 4		35 (8%)
Procalcitonin (PCT) (ng.mL^{-1})	462	0.25 (0.11–1.14)
PCT > 0.25		236 (51%)
PCT > 2		88 (19%)
nSIRS Criteria	462	
0		73 (16%)
1		133 (29%)
2		153 (33%)
3		81 (17%)
4		22 (5%)

Data are expressed as mean ± SD, median [25–75% Interquartile, IQR], or number (percentage).

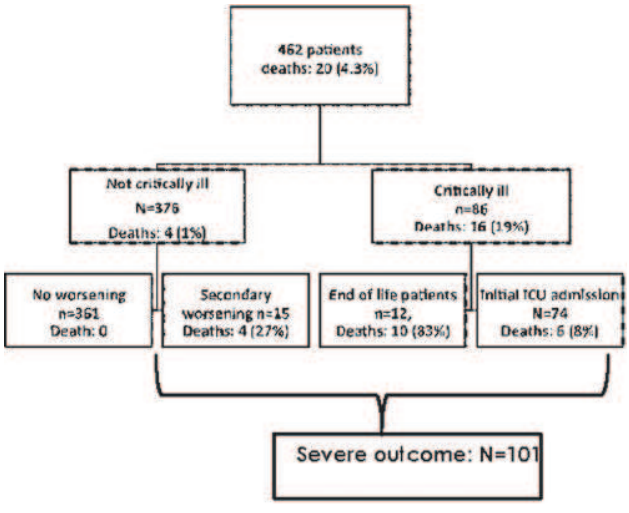

Figure 1. Study flow chart (n = 462).

Table 2. Area under ROC curves (AUC_{ROC}) of lactate, PCT and number of SIRS criteria (nSIRS) for severe outcome, sepsis, severe sepsis and septic shock.

End point and variables	Threshold	AUC_{ROC} [95% CI]	*p* value
Severe outcome			
Lactate ($mmol.L^{-1}$)	2.0	0.679 [0.604–0.731]	<0.001
PCT ($ng.mL^{-1}$)	0.80	0.664 [0.594–0.724]	<0.001
nSIRS (*n*)	2	0.605 [0.545–0.659]	<0.001
Sepsis			
Lactate ($mmol.L^{-1}$)	1.4	0.565 [0.508–0.616][a,b]	0.02
PCT ($ng.mL^{-1}$)	0.25	0.748 [0.701–0.788][a]	<0.001
nSIRS (*n*)	2	0.678 [0.625–0.722][b]	<0.001
Severe sepsis			
Lactate ($mmol.L^{-1}$)	2.0	0.792 [0.736–0.838][a]	<0.001
PCT ($ng.mL^{-1}$)	0.5	0.722 [0.659–0.775][a]	<0.001
nSIRS (*n*)	2	0.638 [0.582–0.688][b]	<0.001
Septic shock			
Lactate ($mmol.L^{-1}$)	2.60	0.840 [0.719–0.912][a]	<0.001
PCT ($ng.mL^{-1}$)	0.60	0.865 [0.737–0.933][a]	<0.001
nSIRS (*n*)	2	0.675 [0.573–0.757][b]	<0.001

p value refers to the comparison vs 0.50 (i.e. no discrimination).
[a]$p < 0.05$ vs nSIRS.
[b]$p < 0.05$ vs PCT.

2 unexpected deaths and 13 secondary admissions to ICU (including 4 septic shocks).

On the 462 patients included, 90 (19%) had co-morbidities and/or treatments that could contribute to raised lactate levels, comprising 43 patients with cancer, 10 with alcoholic intoxication, 12 on β-2 agonist treatment, 7 with HIV infection, 6 with diabetes mellitus, 4 with anemia, 4 cases of shock of other origin than sepsis, and 5 patients with seizures.

Prediction of sepsis, severe sepsis, septic shock and severe outcome

The performances of PCT, lactate and number of SIRS criteria were evaluated according to the area under ROC curve (Table 2 and Figure 2). Although PCT appeared more effective in predicting sepsis (threshold: 0.25 ng/mL), lactate was superior in identifying severe sepsis or severe outcome (threshold: 2.0 mmol/l) and was equivalent to PCT in predicting septic shock. For each clinical group studied, the number of SIRS criteria performed less well than PCT and lactate.

Multivariate analysis showed that PCT was the best independent variable to identify sepsis while lactate was the best for the diagnosis of severe sepsis (Table 3). PCT and lactate performed similarly to identify septic shock but less well than systolic blood pressure (SBP) <90 mmHg (Table 3). Finally, severe outcome was more appropriately identified by clinical variables (SBP < 90

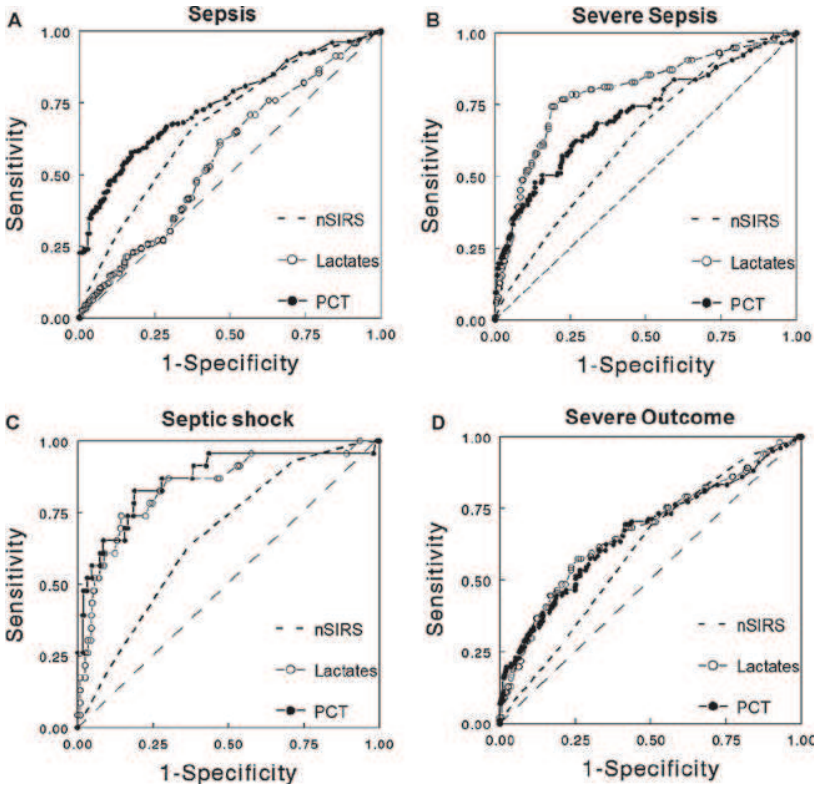

Figure 2. Receiver operating characteristic (ROC) curves of procalcitonin (PCT), lactate and the number of SIRS criteria (nSIRS) for the diagnosis of sepsis (A), severe sepsis (B), septic shock (C) and severe outcome (death or ICU admission during hospital course) (D). The dotted line is the identity line (no discrimination).

Table 3. Variables independently associated with severe outcome (death or ICU admission during hospital course), sepsis, severe sepsis and septic shock.

Clinical group and variables	OR [95% CI]	*p* value
Severe outcome		
SAP < 90 mm Hg	7.13 [2.58–19.69]	<0.001
SpO2 < 90%	3.32 [1.84–5.99]	<0.001
Lactate > 2 mmol.L^{-1}	2.95 [1.76–4.94]	<0.001
Creatinine > 120 µmol.L^{-1}	2.95 [1.70–5.15]	<0.001
PCT > 0.80 ng.mL^{-1}	1.73 [1.02–2.94]	0.04
Sepsis		
PCT ≥ 0.25 ng.mL^{-1}	3.98 [2.60–6.10]	<0.001
Temperature > 38 or < 36°C	2.42 [1.47–3.98]	<0.001
WBC count > 12,000.mm^{-3}	1.83 [1.17–2.86]	0.008
Severe sepsis		
Lactate > 2 mmol.L^{-1}	10.88 [6.51–18.19]	<0.001
PCT≥ 0.25 ng.mL^{-1}	4.42 [2.59–7.54]	<0.001
Septic shock		
SAP < 90 mm Hg	14.44 [4.34–48.05]	<0.001
Lactate > 2 mmol.L^{-1}	6.36 [1.87–21.62]	0.003
SpO2 < 90%	4.99 [1.62–15.35]	0.005
PCT > 0.80 ng.mL^{-1}	6.71 [1.99–22.69]	0.002

Multivariate analysis. Data are expressed as odds ratios (OR) and their 95% confidence interval [95% CI].
nSIRS, number of SIRS criteria; SAP, systolic arterial pressure; SpO2, peripheral pulse oximetry; WBC, white blood cell.

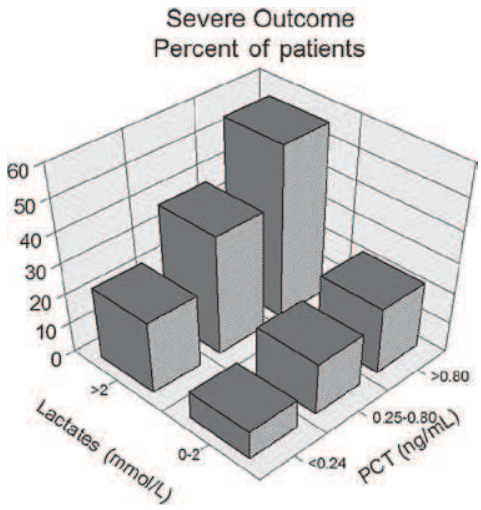

Figure 3. Percentage of patients with a severe outcome (death or ICU admission during hospital course) according to the presence of elevated lactate and/or procalcitonin ($n = 462$, $\chi^2 = 59.1$, $p < 0.001$).

mmHg and pulse oximetry <90%) although PCT, lactate and high creatinine levels remained independent predictive variables, PCT exhibiting the lowest odd ratio (Table 3). The respective contribution of PCT and lactate (according to their serum level) for the prediction of severe outcome is shown in Figure 3. Lastly, we calculated main diagnostic variables associated with an elevated PCT (>0.8 mg/L), an elevated blood lactate (>2 mM/L), one of these two variables, and both of them in predicting severe outcome (Table 4).

Discussion

The accurate identification and risk-stratification of infected patients is of major concern in emergency room, in order to implement a targeted therapy as soon as possible (Rivers et al. 2001). Apart from efforts to identify potential source of infection and recording vital parameters and clinical signs, emergency physicians may use biological tools to improve their clinical judgment. Such biological parameters may either reflect hemodynamic consequences of sepsis (such as blood lactate measurement) or the systemic host response to bacterial invasion (such as serum PCT level). In the present study, we report the respective usefulness of lactate and PCT measurements for the diagnosis and risk-stratification of patients suspected of having sepsis who present to the ED. Only 61% of patients suspected of infection had sepsis, which requires some comments. Indeed, due to the large polymorphism and sometimes cryptic presentation of infected patients at the emergency department, emergency physicians have to favor sensitivity rather than specificity. Unsurprisingly, PCT appeared to perform better for the diagnosis of sepsis while lactate was slightly more predictive of critical illness (Tables 2, 3, and 4, Figure 2). Both biological variables were predictive of severe outcome, defined as death or ICU admission during hospital course, although PCT performed less than other biological variables as creatinine (Table 3). Our data confirm that SIRS criteria are neither sensitive nor sufficiently specific (Levy et al. 2003). Rather than competing, lactate and PCT provided complementary informations on outcome. Therefore, a patient having both a lactate level above 2 mmol.L^{-1} and a PCT above 0.8 ng.mL^{-1} had an enhanced risk of severe outcome (56%) compared to patients having only one of these biomarkers raised (21.7% for PCT and 23.8% for lactate) or any (9.2%) (Figure 3, Table 4).

The prognostic value of the serum lactate level in patients admitted to the ED for a suspected infection is now well-established (Shapiro et al. 2005, Howell et al. 2007, Mikkelsen et al. 2009, Vorwerk et al. 2009) and remains of value even in patients without obvious hypoperfusion and/or organ dysfunction (Howell et al. 2007, Mikkelsen et al. 2009). The lack of early lactate clearance at 6 h seems to be more useful in predicting poor prognosis than the baseline lactate value probably because it reflects non-optimal hemodynamic resuscitation (Nguyen et al. 2004, Arnold et al. 2009, Nguyen et al. 2010). This may be particularly useful for accurately risk-stratifying septic patients who are not immediately candidates for ICU admission. Conversely, as many non-septic conditions (notably seizures) cause raised lactate levels, such results should not lead to a misdiagnosis of severe sepsis state (Fall & Szerlip 2005). Indeed, 19% of our patients had concomitant characteristics that may have contributed to high lactate levels. Therefore, beside the outcome value of lactate measurement there is a place for a sepsis diagnosis biomarker for patients suspected of infection but without obvious clinical focus. PCT has been established as a biomarker of bacterial infection (Hausfater et al. 2002,

Table 4. Diagnostic variables associated with an elevated procalcitonin (PCT > 0.8 mg/L), an elevated blood lactate (>2 mM/L), one of these two variables, and both of them in predicting severe outcome (death or ICU admission during hospital course).

	Sensitivity	Specificity	PPV	NPV	PLR	NLR
Elevated lactate	0.54 [0.45–0.64]	0.76 [0.72–0.81]	0.39 [0.32–0.47]	0.86 [0.81–0.89]	2.31 [1.78–2.98]	0.59 [0.47–0.53]
Elevated PCT	0.51 [0.41–0.60]	0.75 [0.70–0.79]	0.35 [0.28–0.44]	0.84 [0.80–0.88]	2.00 [1.53–2.59]	0.66 [0.53–0.80]
Elevated lactate and/or PCT	0.72 [0.63–0.80]	0.58 [0.53–0.63]	0.33 [0.27–0.39]	0.88 [0.84–0.92]	1.74 [1.45–2.06]	0.47 [0.33–0.54]
Elevated lactate and PCT	0.33 [0.24–0.42]	0.93 [0.90–0.95]	0.56 [0.42–0.69]	0.83 [0.79–0.87]	4.54 [2.86–7.27]	0.73 [0.62–0.82]

Data are expressed as values and their 95% confidence interval [95% CI].
NLR, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; PLR, positive likelihood ratio.

Hausfater et al. 2007) and there is growing evidence for its usefulness as an indicator for starting or stopping antibiotics, notably in lower respiratory tract infections (Christ-Crain et al. 2004, Arnold et al. 2009, Bouadma et al. 2010). The outcome predictor value of PCT in sepsis, although remaining controversial, has been reported in the ICU and ED settings (Hausfater et al. 2002, Clec'h et al. 2004, Hausfater et al. 2007, Phua et al. 2008, Viallon et al. 2008). In 72 patients with septic shock, Phua, Koay and Lee studied the prognostic value of lactate, PCT and several cytokine levels (from day 1 to day 3 following ICU admission) and reported that elevated baseline lactate levels exhibited superior prognostic accuracy than baseline PCT levels, although both remained inferior to baseline cytokine levels and APACHE II and SOFA scores (Phua et al. 2008). This is in accordance with our results as PCT and lactate levels were independent variables associated with severe outcome but performed less well than systolic blood pressure and pulse oximetry (Table 3). However, the clinical context is quite different between patients already admitted to the ICU with the highest level of care (Phua et al. 2008) and patients in the ED being evaluated and risk-stratified for suspected infection. In other words, without questioning the fundamental role of clinical variables in evaluating septic patients, having the use of biological data with added-value for prognosis may be of particular value for the physician to warn or highlight the potential severity of the infection. To date, lactate and PCT measurement appeared to be the best candidates as cytokine levels are not routinely performed and still controversial (Lvovschi et al. 2011). Recently, Green et al. studied the contribution of C-reactive Protein (CRP) stratification to lactate levels for the prognosis of patients admitted through the ED for suspected infection, and found that patients with both a lactate level greater than or equal to 4.0 mmol.L^{-1} and a CRP greater than 10.0 mg.dL^{-1} had an increased risk of short-term mortality (Green et al. 2011). Although we did not study CRP in the current study, due to the enhanced specificity of PCT for bacterial infection and its close relation to severity, we think that PCT may be more suitable than CRP when measured together with lactate for risk-stratification of septic patients (Hausfater et al. 2002, 2007, Claeys et al. 2002, Simon et al. 2004, Claessens et al. 2010a, 2010b).

Several limitations of our study should be noted. First, since the criteria of inclusion specified the availability of both PCT and lactate measurements, we cannot confirm that all patients with sepsis were indeed taken into account. However, since lactate and PCT levels are part of normal practice in our ED when caring for patients suspected of being infected (Hausfater et al. 2002, Hausfater et al. 2007), we think that most of our septic patients over a period of one year have been included. Secondly, as we took into account hyperlactatemia as an already relevant criteria for severe sepsis definition (Levy et al. 2003), this should have overestimated the diagnostic properties of lactate. Thirdly, this was a single-center study, the results may not be applicable to other EDs. Fourthly, it cannot be excluded that the knowledge of baseline lactate and PCT results by emergency physician affected some diagnostic decisions, which was unavoidable in this observational study. Finally, the subgroup with secondary worsening was not large enough (15 patients) to allow statistical analysis of the predictive variables, although this was potentially the most clinically-relevant group of interest. Additional large scale studies are needed to explore this particular subgroup of patients.

Conclusions

For patients evaluated in the ED for suspected infection, the combination of lactate and PCT measurements together with clinical data and vital variables provide complementary informations for diagnosis and risk-stratification. Patients with lactate above 2 mmol.L^{-1} and a PCT above 0.8 ng.mL^{-1} may be at highest risk for severe outcome.

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II) Article 2 : copeptine et troponine dans le syndrome coronaire aigu

A. Introduction

L'association de deux biomarqueurs aux urgences a été décrite pour la première fois dans les années 1990, pour le diagnostic rapide du Syndrome Coronaire Aigu (SCA) [57,58]. L'approche multimarqueurs s'est avérée nécessaire du fait du délai d'environ 3 heures après le début de la douleur thoracique pour identifier une élévation de la troponine conventionnelle. Le SCA est une pathologie dont la prise en charge rapide est indispensable, et reste grevé d'une lourde morbi-mortalité. L'objectif aux urgences est d'exclure de manière certaine ce diagnostic, et donc de privilégier la sensibilité et la valeur prédictive négative (VPN) pour la stratégie diagnostique. La mesure de la troponine Ic à l'admission a une VPN insuffisante pour exclure le SCA, et nécessite donc des mesures répétées. Pendant les trois premières heures, d'autres candidats plus sensibles que la troponine ont été décrit, comme la myoglobine ou la Créatine Kinase MB (CK-MB). Déjà suggérée en 1999 par Apple et al. [111], McCord et al. ont décrit une stratégie diagnostique utilisant la combinaison de la troponine et de la myoglobine pour exclure le diagnostic de SCA aux urgences [112]. Leur étude montre l'intérêt d'une stratégie multimarqueurs aux urgences : la VPN de la combinaison myoglobine/troponine atteint 99.6%, quand la VPN respective de chacun de ces marqueurs ne dépasse pas 94% - ce qui est insuffisant pour exclure le diagnostic de SCA aux urgences. Plusieurs études similaires ont confirmé l'intérêt potentiel d'une combinaison de biomarqueurs cardiaques pour réduire le délai nécessaire avant de pouvoir exclure le diagnostic de SCA aux urgences[113–116].

La copeptine, biomarqueur de stress, de cinétique rapide est apparue comme un bon candidat en association avec la troponine pour pouvoir exclure plus rapidement le diagnostic de SCA [117]. Nous avons fait l'hypothèse que le dosage concomitant de la troponine et de la copeptine aux urgences peut permettre une exclusion rapide et fiable du SCA.

B. Discussion

Cette étude prospective multicentrique confirme les résultats suggérés ces dernières années : la combinaison de la Copeptine et de la Troponine permet une amélioration des performances diagnostiques pour le SCA aux urgences. La VPN de la Troponine Ic isolée étant à 95% [92%-97%], elle est insuffisante pour exclure le SCA sur un seul prélèvement. En revanche, l'association avec la Copeptine améliore significativement cette valeur, et la VPN est à 99% [97%-100%]. Ainsi, cette étude observationnelle laisse entrevoir la possibilité d'une stratégie basée sur un seul dosage.

En particulier, nous avons séparé dans cette étude les patients selon leur probabilité pré test (empirique) de SCA, et la combinaison Troponine/Copeptine présente des caractéristiques très prometteuses pour les patients de faible probabilité, avec une sensibilité et une VPN de 100%. Ainsi une stratégie mêlant la probabilité pré test clinique, et une combinaison de biomarqueurs à forte VPN pourrait être adoptée, à l'instar de celle couramment utilisée pour exclure le diagnostic d'embolie pulmonaire [118].

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Copeptin for rapid rule out of acute myocardial infarction in emergency department ☆

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ABSTRACT

Background: Copeptin, in combination with conventional troponin (cTn), has been suggested as a means of rapid rule out of the diagnosis of acute myocardial infarction (AMI). This study aims to assess the value of copeptin for rule out of AMI, according to the pre-test probability (PTP).
Methods: In a prospective multicentric study, we enrolled patients presenting into emergency departments with chest pain <6 h, copeptin was measured, and PTP was quoted. The discharge diagnosis was adjudicated by 2 independent experts using all available data, including cTnI.
Results: 317 patients were included: 148 (46%) had low, 110 (35%) moderate and 59 (19%) high PTP. Final diagnosis was AMI in 45 patients (14%). Median copeptin level was higher in AMI patients compared with that in patients having other diagnoses (23.2 vs. 9.9 pmol/L, $p = 0.01$). A copeptin level ≥10.7 pmol/L in combination with cTnI detected AMI with higher sensitivity than for cTnI alone (98 [87–100] vs. 71 [55–83] %, $p = 0.001$), whatever the PTP. The negative predictive value of the combination copeptin + cTnI was increased, compared to that of cTnI alone (99 [97–100] vs. 95 [92–97] %, $p < 0.05$).
Conclusions: In triage of chest pain patients, the additional use of copeptin with conventional cTnI might allow a rapid and reliable rule out of the diagnosis of AMI regardless of the PTP.

1. Introduction

Early detection of acute myocardial infarction (AMI) remains suboptimal. Diagnosis of AMI relies, besides clinical symptoms and electrocardiographic (ECG) findings, primarily on biomarker levels. Although quite specific [1], ST-elevation has only a 50 to 60% sensitivity for diagnosis of AMI [2]. Markers of myocardial necrosis such as cardiac troponins (cTn) are the gold standard in detection of AMI, and their use is recommended by current guidelines [3]. In particular, cardiac troponin I (cTnI) provides excellent specificity. However, cTnI does not reliably exclude AMI without repeated negative measurements over 4–6 h, and myoglobin is limited by its poor specificity [4]. Therefore, there is a need for a fast and reliable test to facilitate triage, diagnosis and adequate treatment strategies. This would be of great value in patients presenting with atypical symptoms or a non-contributive ECG. The release of necrosis markers from cardiomyocyte is believed to be delayed, and this might explain the weakness in diagnostic performance of conventional cTn assays shortly after the onset of chest pain. Therefore, markers with a pathophysiologic background independent of cell necrosis might improve the rapid diagnosis of AMI.

C-terminal proVasopressin (copeptin) is secreted stoichiometrically with arginine-vasopressin (AVP) by neurohypophysis [5]. AVP plays a crucial role in the regulation of the hypothalamo-pituitary–adrenal axis, reflecting the individual stress response [6, 7]. The glycosylated peptide copeptin is part of the uncleaved pro-AVP and emerges equimolar to AVP, because both are derived from the precursor prepro-AVP along with neurophysin II; therefore, it serves as an indirect marker for AVP. Direct measurement of AVP is lacking, but a recently developed assay for copeptin delivers the stability and reproducibility [8]. The release pattern of copeptin in AMI patients (an immediate rise after onset of chest pain and decrease toward physiologic levels within 5 days) as well as the potential use of copeptin in rule-out of AMI was described recently [9]. Thus, the role of copeptin as diagnostic marker in suspected AMI needs to be evaluated in large prospective cohorts. Two recent studies strongly suggested that

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combined determination of cTn and copeptin provides a very accurate negative predictive value and therefore aids early and safe ruling-out of AMI [9, 10]. Nevertheless, the interest of biomarkers might be variable according to the pre-test probability (PTP) assessed by the physician [11, 12]. For example, D-dimers measurement is strongly useful for ruling out pulmonary embolism if the PTP is low or moderate, and not indicated if the PTP is high [11]. Thus, our hypothesis was that the usefulness of these association biomarkers of AMI might be different according to the PTP.

The aim of the current study was to determine prospectively whether the sensitivity of combination of copeptin with conventional cTnI was superior to that of cTnI alone in the early diagnosis of AMI in emergency patients, and to evaluate the usefulness of that combination according to the PTP assessment.

2. Patients and methods

2.1. Study population and design

During the period from August 2005 to January 2007 in three academic hospitals we prospectively enrolled consecutive out-hospital patients (>18 years) who presented to the ED with chest pain suggestive of AMI with onset or peak within the last 6 h. The study was performed according to the principles of the Declaration of Helsinki and approved by the local ethics committee (Comité de Protection des Personnes, Pitié-Salpétrière Hospital). Because routine medical care was unchanged, waived informed consent was authorized. In our institutions, the coronarography is performed by cardiologists in STEMI as recommended [3]. However, for NSTEMI, coronarography was left on the cardiologist's in charge.

Patients with terminal kidney failure requiring dialysis were planned to be excluded. However, none of the enrolled patients presented with terminal kidney failure requiring dialysis.

2.2. Routine clinical assessment

As part of the routine assessment in our institutions, all patients underwent an initial clinical evaluation that included clinical history, physical examination, 12-lead electrocardiogram (ECG), pulse oximetry, routine blood tests, and chest X-ray. After these routine tests, and before cardiac biomarker results were revealed, emergency physicians were asked to cote an "empirical" clinical probability of AMI: low, medium or high pre-test probability (PTP) [13]. Because a validated score of AMI (as in pulmonary embolism [11]) does not exist yet, we used an empirical one, based on the type of chest pain, physical examination, and ECG modifications. cTnI was measured at presentation and, if the physician thought it was necessary, measurement was repeated after 3 to 9 h, as long as clinically indicated. Thus, according to the diagnosis of NSTEMI (non ST-elevation MI) or STEMI (ST-elevation MI), the patients were admitted directly to the cardiology unit for further evaluation and treatment or directly to the catheterization laboratory for primary percutaneous coronary intervention. However, the timing and treatment of patients were left to the discretion of the attending physicians according to the suspected diagnosis.

Estimated glomerular filtration rate values (eGFR, in $ml^{-1} \cdot min^{-1} \cdot 1.73\ m^{-2}$) were calculated using the revised [14] Modification of Diet in Renal Disease formula [15].

Emergency physicians in charge were blinded to the results of copeptin and myoglobin, and biologists were blinded to the emergency physicians' diagnosis.

2.3. Adjudicated final diagnosis

All 12-lead admission ECGs were reviewed by experts blinded to the copeptin but not to troponin results. The ECG manifestations indicative of AMI were defined according to recommendations in current guidelines [16]. To determine the causal diagnosis at presentation for each patient, two independent experts (emergency physicians), reviewed all available medical records (including patient history, physical findings, results of laboratory and radiologic testing, ECG, echocardiography, cardiac exercise test, coronary angiography, summary chart at discharge) pertaining to the patient from the time of ED presentation to a 30-day follow-up. If there were diagnostic disagreements, cases were reviewed and adjudicated in conjunction with a third expert (also an emergency physician).

AMI was diagnosed according to the joint European Society of Cardiology/American College of Cardiology/American Heart Association/World Heart Federation Task Force redefinition of MI guidelines [1]. Diagnosis of AMI required a cTnI increase (or a rise/fall pattern) above the 99th percentile, associated with at least one of the following: symptoms of ischemia, new ST-T changes or new Q wave on ECG, imaging of new loss of viable myocardium, or normal cTnI on admission [1]. As the conventional cTnI methods (used routinely in our institutions) do not allow the measurement of 99th percentile with the precision required (see below), AMI was diagnosed on the basis of a cTnI value above the 10%CV level [17]. Unstable angina (UA) was diagnosed in patients with conventional cTnI <10%CV levels and typical angina at rest, a deterioration of a previously stable angina, in cases of cardiac catheterization with coronary arteries found to have stenosis ≥70%, and in ambiguous cases in which follow-up information revealed AMI or a sudden unexpected cardiac death within 30 days. Pre-defined further diagnostic categories included AMI (STEMI with the presence of ST-segment elevation in ≥2 continuous leads on electrocardiography or new onset of left bundle branch block-LBBB, or NSTEMI), unstable angina, and a third group including cardiac but not coronary symptoms (e.g., stable angina, myocarditis, arrhythmias, heart failure) and noncardiac symptoms (e.g., pulmonary embolism…). When no sufficient further diagnostic procedures were performed for conclusive diagnosis, symptoms were classified as being of unknown origin, and included in the third group.

2.4. Biochemical analysis

The goal of the present study was to evaluate the combination of conventional cTnI and copeptin compared to conventional cTnI alone. Thus, we evaluated as comparators the conventional cTnI used routinely in our institutions.

In two EDs (Cochin Hospital and La Pitié Salpêtrière Hospital), plasma cTnI concentrations were routinely measured on an X-pand® HM analyser, using the Cardiac Troponin-I immunoassay (Siemens Healthcare Diagnostics Inc., Newark, New Jersey, USA). The measuring range extended from 0.04 to 40.00 μg/L. The 99th percentile for this method is 0.07 μg/L, with coefficients of variations (CV) between 15 to 22%. The limit of quantitation (i.e. the lowest analyte concentration that can be reproducibly measured with a between-run CV of 10%, or 10%CV) is 0.14 μg/L.

In the Bicêtre Hospital, plasmatic cTnI concentrations were routinely measured on an Access® analyser (Beckman Coulter, Inc., Brea, CA, USA). The measuring range of this immunoassay extended from 0.01 to 100.00 μg/L. The 99th percentile for this method is 0.04 μg/L, and the limit of quantitation (10%CV) announced by the manufacturer is 0.06 μg/L.

Plasmatic myoglobin concentrations were routinely measured on same analyzers than cTn in each hospital. The threshold value used in our study was 90 μg/L (99th percentile for the diagnostic of AMI) [18]. Myoglobin determinations were performed blinded to the clinical assessment of the emergency physicians.

2.5. Copeptin measurement

Copeptin was measured in heparinized samples collected on admission. The assay was performed on a KRYPTOR® analyzer using the commercial sandwich immunoluminometric assay (B.R.A.H.M.S Copeptin KRYPTOR, B.R.A.H.M.S Aktiengesellschaft, Hennigsdorf, Germany). The assay principle lies on TRACE technology (Time-Resolved Amplified Cryptate Emission). The lower detection limit is 4.8 pmol/L, and the functional assay sensitivity (20%CV) is <12 pmol/L. The limit of quantification (10%CV) is 14.1 pmol/L (data from manufacturer). Values <4.8 pmol/L were considered as 4.8 pmol/L. In our laboratory, CV were found to be <5% (4.4% at 28.86 pmol/L and 4.6% at 95.84 pmol/L). Copeptin determinations were performed blinded to the clinical assessment of the emergency physicians.

2.6. Statistical analysis

Continuous variables are presented as mean ± SD or median (25th–75th percentile), categorical variables as numbers and percentages. Continuous variables were compared with the Mann–Whitney *U* test and categorical variables using the Pearson chi-square test. Correlations among continuous variables were assessed with the use of the Spearman rank-correlation coefficient.

Receiver–operator characteristic (ROC) curves were constructed to assess the sensitivity and specificity, positive (PPV) and negative predictive value (NPV), positive and negative likelihood ratio (all with their 95% confidence intervals [95% CI], calculated with the Wilson's score with correction of continuity) throughout the concentrations of cTnI, myoglobin and copeptin, and to compare the ability of these cardiomarkers (alone or in combination) to diagnose AMI. Comparison of areas under the ROC curves was performed as recommended [12]. Since different methods were used for cTnI, the validity of the ROC curve might be discussed. Thus, we performed additional subgroup analyses according to the methods used. As this comparison is recognized to be potentially insensitive, the Net Reclassification Index (NRI) method was used, as recently described [12, 19]. For tests with binary outcomes (such as cardiac markers for the diagnosis of AMI), NRI is the same as the gain in certainty of the first test minus the gain in certainty of the second test, or alternatively stated, the differences of the sum of the sensitivity and specificity: $NRI_{\text{second test vs. first test}} = (\text{Sensitivity} + \text{Specificity})_{\text{second test}} - (\text{Sensitivity} + \text{Specificity})_{\text{first test}}$.

All tests were 2-tailed, and a p value of <0.05 was considered significant. Statistical analysis was performed using StatView 5.0 for Windows (SAS Institute Inc., Cary, USA) and for ROC analysis using MedCalc 10.3.2.0 for Windows (MedCalc Software, Mariakerke, Belgium). Graphs were built with GraphPad Prism 5 (GraphPad Software, La Jolla, USA).

3. Results

3.1. Characteristics of patients

After 18 months, 317 consecutive patients were enrolled in the study. Baseline characteristics of patients according to empirical probability of

AMI (estimated by attending emergency physicians) are shown in Table 1. The mean age was 57 ± 17 years (range: 40–90 years), and 205 (65%) were male. Chest pain was considered typical of acute coronary syndrome in 43% (n = 136) of patients, and onset of chest pain was less than 3 h in 61% (193 patients out of 268).

The adjudicated final diagnosis was AMI in 14% of patients (n = 45), unstable angina in 3% (n = 11), other diagnosis in 82% (n = 261). Of the patients with AMI, 29% (n = 13) were diagnosed having STEMI and 71% (n = 32) as having NSTEMI. Patients with adjudicated other diagnoses included patients with stable angina (n = 23), myopericarditis (n = 44), pulmonary embolism (n = 16), ACFA (n = 8), hypertensive crisis (n = 6), heart failure (n = 5), and tachycardia (n = 3). Of note, we did not have any Takotsubo cardiomyopathy in our cohort. Compared to patients with other adjudicated diagnoses patients with a final diagnosis of AMI were older (63 ± 17 vs. 56 ± 17 years, p = 0.017), and had coronarography more frequently during hospitalization (78% vs. 18%, p<0.001) were treated more frequently with aspirin (80% vs. 31%, p<0.001), clopidogrel (49% vs. 11%, p<0.001), and low molecular weight heparin (58% vs. 15%, p<0.001). Patients (n = 45) with confirmed AMI were also more frequently hospitalized (98% vs. 54%, p<0.001), and presented more frequently with a positive cTnI (69% vs. 3%, p<0.001), positive myoglobin (42% vs. 14%, p<0.001), and lower median eGFR (76 vs. 77 $ml^{-1} \cdot min^{-1} \cdot 1.73\ m^{-2}$, p = 0.041) at admission. Of the 45 patients with AMI, 10 (22%) patients did not undergo a coronarography. They presented more frequently a creatinine clearance less than 50 mL/min (40% vs. 3%, p = 0.006). One of them had STEMI; she was a 95 year old woman with an eGFR at 49 mL/min/1.73 m^2, a concomitant urinary tract infection and a poor functional status.

In our 317 patients, 149 (47%) were empirically quoted low PTP of AMI, 117 (37%) moderate, and 51 (16%) high PTP. Table 1 shows that the three groups of patients differed in numerous clinical characteristics, regarding their empirical PTP.

3.1.1. Characteristics of patients with a single measurement of troponin

Patients who did not undergo a second troponin measurement (n = 207, 65% of the study population) were: all STEMI patients (n = 12), 31 NSTEMI patients (in whom, 26 had at admission a positive conventional cTnI), 8 UA patients (all had admission cTnI negative), and 154 patients with other diagnosis (in whom 147 had at admission a negative cTnI). Patients with other diagnosis and a positive cTnI at admission (n = 7) had: pericarditis (n = 2), tachycardia, AVC, pulmonary embolism (n = 2), pneumopathy.

3.2. Copeptin's diagnostic performances

Median copeptin levels (as well as cTnI and myoglobin levels) were significantly higher in patients with AMI than in patients with other adjudicated diagnoses (23.2 pmol/L vs. 9.9 pmol/L, p = 0.01) (Fig. 1).

3.2.1. ROC analysis

The highest area under the ROC curve (AUC) for the diagnostic of AMI was for initial cTnI (AUC of Siemens cTnI assay = 0.93 [0.87–0.98],

Table 1
Baseline characteristics of the population according to the empirical pre-test probability (PTP) of AMI.

	All patients	Patients according to PTP			p[a]
		Low	Moderate	High	
n	317	148	110	59	
Age (years)	57 ± 17	53 ± l18	61 ± 16	60 ± 17	0.0005
Men	205 (65)	88 (59)	78 (71)	39 (66)	0.158
Mean systolic BP (mm Hg)	141 ± 28	135 ± 24	148 ± 29	144 ± 30	0.0008
Mean diastolic BP (mm Hg)	80 ± 16	78 ± 15	83 ± 18	82 ± 16	0.061
Mean heart rate	85 ± 45	86 ± 23	82 ± 22	80 ± 19	0.126
Mean SpO_2 (%)	97 ± 4	97 ± 4	97 ± 2	97 ± 2	0.639
Familial history of CAD	100 (32)	26 (18)	51 (44)	23 (39)	<0.0001
Personal history of CAD	83 (26)	12 (8)	44 (40)	27 (46)	<0.0001
Dyslipidemia	113 (36)	28 (19)	58 (53)	27 (46)	<0.0001
Smoking	128 (40)	50 (34)	49 (45)	29 (49)	0.071
Diabetes	44 (14)	9 (6)	22 (20)	13 (22)	0.0008
Hypertension	116 (37)	35 (24)	54 (49)	27 (46)	<0.0001
History of heart failure	21 (7)	4 (3)	10 (9)	7 (12)	0.025
Typical chest pain	136 (43)	56 (38)	49 (45)	31 (53)	0.137
Chest pain onset <3 h	193 (61)	90 (61)	67 (61)	36 (61)	0.670
Chest pain onset >3 h	75 (24)	31 (21)	27 (25)	17 (29)	
Patients with positive Tn Ic at admission[b]	41 (13)	6 (4)	18 (16)	17 (29)	<0.0001
Patients with positive myoglobin at admission (>90 μg/L)	56 (18)	14 (9)	18 (16)	24 (41)	<0.0001
Median eGFR ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	77 (62–94)	81 (67–98)	71 (61–90)	76 (56–91)	0.007
TIMI score	1 (0–3)	0 (0–1)	2 (1–3)	2 (1–4)	<0.0001
Coronarography	83 (26)	20 (14)	31 (28)	32 (54)	<0.001
Final diagnosis of AMI	45 (14)	4 (3)	18 (16)	23 (39)	<0.0001
STEMI	12 (4)	0 (0)	0 (0)	12 (20)	0.0004
NSTEMI	33 (10)	4 (3)	18 (16)	11 (19)	
Final diagnosis of UA	11 (3)	0 (0)	4 (3)	7 (14)	<0.0001
Other diagnosis[c]	261 (82)	144 (97)	88 (75)	29 (57)	<0.0001
Hospital-admission	192 (61)	66 (45)	74 (67)	52 (88)	<0.0001
Admission in ICU	138 (44)	35 (24)	53 (48)	46 (78)	<0.0001
Treatment received in the first 24 h of admission					
Aspirin	119 (38)	26 (18)	53 (48)	40 (68)	<0.001
Clopidogrel	54 (17)	7 (5)	22 (20)	25 (42)	<0.001
LMWH	68 (21)	13 (9)	26 (24)	27 (46)	<0.0001
Anti-GPIIb/IIIa	3 (1)	1 (1)	0 (0)	2 (3)	0.085

ACS, acute coronary syndrome; AMI, acute myocardial infarction; UA: unstable angina; BP, blood pressure; CAD, coronary acute disease; eGFR, estimated glomerular filtration rate; ICU, Intensive car unit; LMWH, low molecular weight heparin; NSTEMI, non ST elevated myocardial infarction; PTP, pre-test probability; STEMI, ST elevated myocardial infarction. TIMI, Thrombolysis in Myocardial Infarction. Results are in mean ± SD, median (25th–75th percentile), or number (percentage).

[a] Across PTP groups.

[b] >0.14 μg/L in PSL and CCH, >0.06 μg/L in KB.

[c] Including: stable angina (n = 23), myopericarditis (n = 44), pulmonary embolism (n = 16), ACFA (n = 8), hypertensive crisis (n = 6), heart failure (n = 5), tachycardia (n = 2).

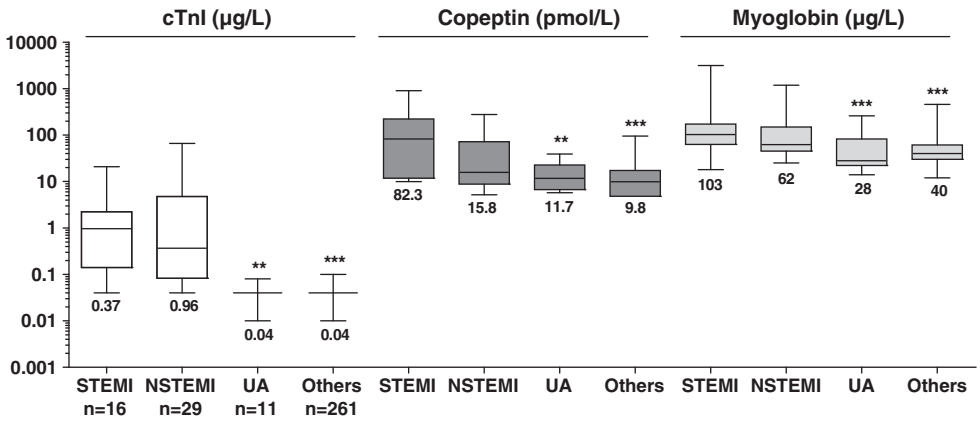

Fig. 1. Box plots for cTnI, copeptin and myoglobin values according to final diagnosis. **p<0.01 vs. AMI patients (STEMI + NSTEMI); ***p<0.001 vs. AMI patients (STEMI + NSTEMI). cTnI: cardiac troponin I, STEMI: ST elevation myocardial infarction, NSTEMI: non ST elevation MI, UA: unstable angina. White boxes: cTnI, gray boxes: copeptin, and dotted boxes: myoglobin. Medians are indicated for each box.

p<0.001; AUC of Beckman cTnI assay = 0.97 [0.94–1.00], p<0.001; NS between AUC of cTnI assays), compared to a lower AUC with copeptin (AUC = 0.731 [0.651–0.811], p = 0.002), and myoglobin (AUC = 0.728 [0.646–0.809], p<0.001). AUCs of copeptin and myoglobin were not significantly different, but both were significantly lower than that of cTnI (p<0.001 vs. AUC of Siemens cTnI, p<0.05 vs. AUC of Beckman cTnI). ROC analysis indicated an optimal copeptin threshold for the diagnosis of AMI at 10.7 pmol/L (sensitivity 81% [66–91], specificity 53% [47–59], PPV 21% [9–34], NPV 95% [92–97], accuracy 57% [51–62]). One hundred and sixty one patients (51%) presented with a copeptin level >10.7 pmol/L. This proportion was lower in the low PTP group (43%) than in the moderate PTP group (55%) or than in the high PTP group (62%) (p across PTP groups = 0.027). Furthermore, patients with AMI presented more often a copeptin >10.7 pmol/L in comparison with other patients (82% vs. 46%, p<0.001).

Performing the analysis without STEMI patients, we observed similar results than above. The highest AUC for the diagnostic of AMI was for initial cTnI (AUC = 0.940 [0.907–0.965], p<0.001), compared to a lower AUC with copeptin (AUC = 0.702 [0.646–0.754], p<0.001), and myoglobin (AUC = 0.705 [0.650–0.757], p<0.001). AUCs of copeptin and myoglobin were not significantly different, but both were significantly lower than that of cTnI (p<0.001). ROC analysis indicated an optimal copeptin threshold value for the diagnosis of AMI at 10.6 pmol/L.

3.2.2. Combination of cTnI with copeptin or myoglobin

Combination of a positive cTnI and/or a positive copeptin demonstrated to significantly increase sensitivity and NPV in comparison to cTnI alone, in all patients regardless of the PTP (Table 2). Thus, sensitivity and NPV were significantly increased in high PTP patients, when copeptin was combined with cTnI. Combination of cTnI with myoglobin failed to be effective in this analysis.

3.2.3. Patients with second measurement of cTnI

Some patients (n = 110, i.e. 40% of patients with negative cTnI at admission) had a second measurement of cTnI 3 to 9 h after admission, because the first cTnI was not elevated. In the low PTP group, 1 patient (out of 4) with AMI presented a cTnI negative at admission. This patient had also a negative second cTnI, but the copeptin level appeared to be positive (71.9 pmol/L) at admission. In the moderate PTP group, 4 patients (out of 18) with AMI presented with a negative cTnI at admission. Only one patient had a second measurement which was positive for cTnI and the copeptin level appeared to be positive (10.74 pmol/L) at admission. The other 3 patients did not have a second measurement, but all of them presented with a positive copeptin level. Finally, in the high PTP group, 8 patients (out of 23) with AMI presented a negative cTnI at admission. One patient had a second measurement which was positive for cTnI. Copeptin appeared to be positive (11.1 pmol/L) at admission for this patient. The other seven patients did not have a second measurement, but presented all a positive copeptin (from 10.9 to 231.3 pmol/L). In the sub-group of patients with a second measurement of cTnI (n = 110), and whatever the PTP, the negative predictive value (NPV) of the combination of cTnI and copeptin at admission was 100% [93–100], but not significantly different than the NPV (99% [94–100]) of serial measurements of cTnI (at admission and after 3–9 h).

3.3. Reclassification

We further investigated the combination of cTnI with copeptin or with myoglobin. The Net Reclassification Indexes were calculated. In all patients, cTnI alone could identify 32 AMI patients. This adequate classification was increased when combining with myoglobin (34 patients) or with copeptin (44 patients). Conversely, cTnI alone was positive in 9 non-AMI patients, and this number was increased when combining with myoglobin (32 patients) or with copeptin (144 patients). Thus, NRIs for the combination of cTnI with myoglobin (NRI = −7.3 [−0.4 to −14.9] %, p = 0.067), or with copeptin (NRI = −16.0 [−8.2 to −23.6] %, p = 0.0001), indicate that there was no gain in certainty using the combination of cTnI with copeptin, in comparison to cTnI alone. We observed the same regardless of the PTP groups.

4. Discussion

For patients presenting with chest pain in the ED, the major concern for physicians is the ability to rule out AMI as quickly as possible. Our multicentric prospective study involving unselected patients presenting to the ED within 6 h of chest pain suggestive of AMI, examined the value of a dual marker strategy using cTnI, a marker of cardiac necrosis, and copeptin, a marker of endogenous stress, for rapid rule out of AMI. We report three major findings. First, the combination of copeptin, measured by the Kryptor method, with cTnI had a significantly higher sensitivity than cTnI alone (98% vs. 71%), for the diagnosis of AMI. Second, the combination of cTnI with copeptin resulted in a higher diagnostic performance for ruling out AMI on admission (higher sensitivity and NPV), compared to that of a combination of cTnI with myoglobin. However, according to its low specificity, copeptin could not replace cTnI as an indicator of AMI. Third, the additional value of copeptin did not differ according to PTP of AMI evaluated by physicians' in charge. Thus, even in high PTP, the sensitivity and NPV of this association remained almost perfect.

Two recent studies evaluated the diagnostic accuracy of copeptin in combination with cTn in patients with suspected AMI. In a study performed in a monocentric ED, Reichlin et al. [9] reported that copeptin and cTn resulted in a almost perfect AUC for rule-in AMI (0.97), higher than the 0.86 of cTn alone. However, they did not compare to a

Table 2
Diagnostic information of various combinations of cardiac biomarkers for the diagnosis of AMI according to pretest probability (PTP).

	Positive cTnI	Positive cTnI[a] and/or myogoblin[b]	Positive cTnI[a] and/or copeptin[c]
In all patients (n = 317)			
Sensitivity (%)	71 [55–83]	76 [60–87]	98 [87–100][d]
Specificity (%)	97 [94–98]	85 [80–89][d]	54 [46–62][d]
PPV (%)	78 [62–89]	45 [34–57][e]	26 [20–33][d]
NPV (%)	95 [92–97]	96 [91–98]	99 [97–100][e]
Accuracy (%)	93 [90–96]	84 [79–87][d]	60 [55–66][d]
In low PTP group (n = 148)			
Sensitivity (%)	75 [22–99]	75 [22–99]	100 [40–100]
Specificity (%)	98 [94–100]	91 [84–95][f]	60 [52–68][g]
PPV (%)	50 [14–86]	19 [5–46]	7 [2–17][f]
NPV (%)	99 [96–100]	99 [95–100]	100 [95–100]
Accuracy (%)	97 [93–99]	91 [84–95][f]	61 [52–69][g]
In moderate PTP group (n = 110)			
Sensitivity (%)	78 [52–93]	78 [52–93]	94 [71–100]
Specificity (%)	96 [92–100]	84 [74–90][h]	47 [36–57][i]
PPV (%)	78 [59–97]	48 [30–67]	26 [16–38][i]
NPV (%)	96 [92–100]	95 [87–98]	98 [87–100]
Accuracy (%)	93 [88–98]	83 [74–89][h]	55 [45–64][i]
In high PTP group (n = 59)			
Sensitivity (%)	65 [43–83]	74 [51–89]	100 [82–100][j]
Specificity (%)	94 [80–99]	64 [46–79][j]	50 [33–67][k]
PPV (%)	88 [62–98]	57 [38–74][j]	56 [40–71][j]
NPV (%)	81 [65–91]	79 [60–91]	100 [78–100][j]
Accuracy (%)	83 [71–91]	68 [54–79]	70 [56–81]
In high PTP group without STEMI patients (n = 47)			
Sensitivity (%)	82 [48–97]	82 [48–97]	100 [68–100]
Specificity (%)	94 [80–99]	64 [46–79][l]	50 [33–67][m]
PPV (%)	82 [48–97]	41 [22–63][l]	38 [21–58][l]
NPV (%)	94 [79–99]	92 [73–99]	100 [78–100]
Accuracy (%)	92 [79–97]	68 [53–81][l]	62 [46–75][l]

NPV: positive predictive value; NPV: negative predictive value. Values are expressed as a percentage.
[a] > 0.14 μg/L in PSL and CCH, > 0.06 μg/L in BCT.
[b] > 90 μg/L.
[c] > 10.7 pmol/L.
[d] p < 0.001 vs. positive cTnI in all patients.
[e] p < 0.05 vs. positive cTnI in all patients.
[f] p < 0.05 vs. positive cTnI in low PTP group.
[g] p < 0.001 vs. positive cTnI in low PTP group.
[h] p < 0.05 vs. cTnI in moderate PTP group.
[i] p < 0.001 vs. positive cTnI in moderate PTP group.
[j] p < 0.05 vs. cTnI in high PTP group.
[k] p < 0.001 vs. positive cTnI in high PTP group.
[l] p < 0.05 vs. cTnI in high PTP group without STEMI patients.
[m] p < 0.001 vs. positive cTnI in high PTP group without STEMI patients.

combination cTn with myoglobin which until now is still widely used as an early biomarker of AMI, even if cTn is considered as the biomarker of choice for the plasmatic diagnosis of AMI. In specific chest pain units, Keller et al. [10] confirmed that combined copeptin and cTn improved the c-statistic from 0.84 for cTn alone to 0.93. However, the higher rate of confirmed AMI (22%) compared to that usually found in an unselected ED (15%) [20, 21] may be not adapted for unselected ED. Furthermore, none of these previous studies evaluated the various diagnostic performance of copeptin according to the PTP of AMI. Estimation of the clinical probability is usually the first step before choosing the best test (imaging or biomarker) to perform. Thus, it is widely accepted that emergency physicians could exclude pulmonary embolism in low or medium PTP of pulmonary embolism with a high sensitive D-dimers assay [11]. Although emergency physicians used an empirical clinical PTP without validation (see limits below), the outcomes and final diagnosis were significantly different according to their PTP. Thus, we suggested that, even in high risk patients, the combination of conventional cTnI and copeptin could help clinicians for a rapid and safe exclusion of AMI.

In our study, we found a lower threshold for copeptin than previously observed by Reichlin et al. [9]. However, these authors used a different — and more sensitive — method for measuring copeptin. As far as we are aware, their method widely described in the literature, needs 2 h of incubation, and thus is not suitable for emergency practice [22, 23]. However, our value of 10.7 pmol/L was similar to that observed by Keller et al. [10], using a well-adapted 24 h measurement with a result in less than 45 min.

The combinations of marker of myocardial infarction (cTn) with other biochemical markers (that reflect important upstream processes in the physiopathology of AMI) have been studied by several authors. Apple et al. reported an evaluation of a multimarker approach for early diagnosis of AMI [24]. They showed that none of the tested markers (such as myeloperoxydase, soluble CD40 ligand, and matrix metalloproteinase 9) provided any clinically significant additional diagnostic performance when measured in addition to cTnI. In this work, non-necrosis markers were disappointing because of their poor sensitivity. Lindahl et al. gave recently some arguments to explain this failure of multimarker approach in providing additional diagnostic information: cTn alone already has a high diagnostic accuracy [25]. However, because of the excellent NPV of its combination with cTn, copeptin seems to be a good candidate added to cTn for exclusion of AMI in the setting of triage in the ED. Of note, NRI indicates that there is no gain in certainty using the combination of cTnI with copeptin.

5. Limits

We are aware that our study presents several limitations.

Because our study was observational and took place in three different institutions without dedicated chest pain unit, the management could not be homogeneous and not all patients with negative cTnI had second cTnI measurement during their stay in the ED (see above). Thus, the recommended change criteria were not systematically used for all patients. As a consequence, we may have (1) underestimated the rate of AMI, which could partially explain the low percentage of unstable angina (3%) compared to that of previous studies [20, 26], and (2) overestimated the high accuracy of cTn for AMI at presentation.

Second, we used two different assays for cTnI as the comparator. We thus could not combine the cTnI values for building a single ROC curve. However, we observed comparable AUCs, even after combination of cTnI with copeptin or with myoglobin.

Although the enrolment was based on a multicentric recruitment, our study is limited by the number of patients according to PTP sub-groups. Its results, therefore, are preliminary and need to be confirmed and extended. We classified our population according to an empirical clinical PTP without any standardization. However, another empirical classification has already been used by other authors [13] without any accurate validation. In all cases, even in the high PTP group, our results demonstrated a high NPV for the diagnosis of AMI when cTnI and/or copeptin were negative at admission. However, this sub-group (high PTP of AMI) represented only 16% of our population. Thus, further studies should evaluate combination of copeptin with cTnI in a specific sub-group of emergency patients with a high of AMI. We could not demonstrate the usefulness of copeptin in the prediction of adverse events, because we evaluated only its diagnostic performance. Further studies should investigate the usefulness of copeptin in predicting outcome and/or with intervention studies to demonstrate that the use of this biomarker may improve the management of patients with AMI patients in the setting of the emergency room. The method used for measuring cTnI was not a highly sensitive assay, unlike more recent techniques [20, 21, 26], because we used the routine conventional method of our institutions (and in most of all hospital so far). However, a pilot study recently indicated that combining copeptin to highly sensitive cTnT might be efficient for the detection of ACS at admission [27].

6. Conclusion

In triage of chest pain patients, the additional use of copeptin with conventional cTnI may allow a rapid and reliable rule out of the diagnosis of AMI, regardless of the PTP. Associated with cTn, sensitivity and NPV for the diagnosis of AMI were very high, even in patients with high PTP. Further intervention studies should confirm whether a negative single assay of copeptin and cTnI could help triage allowing early discharge of patients with chest pain.

Abbreviations

cTn	cardiac troponine
AMI	acute myocardial infarction
PTP	pre-test probability
AVP	arginine-vasopressin

Conflict of interest

CCG, SG, BR and PR received honoraria from B.R.A.H.M.S.

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III) Article 3 : S100B et Copeptine dans la crise convulsive aux urgences

A. Introduction

Les consultations pour convulsions représentent de 0.5 a 7% [119–122] des visites aux urgences (1 million par an aux USA) [123]. 5 à 10% de la population générale souffrira d'une crise convulsive (CC) au cours de sa vie [124–126] . En prenant en charge une consultation liée à une convulsion, l'urgentiste doit prendre en compte de multiples paramètres pour évaluer le risque de complication et de récidive précoce. La stratégie diagnostique, les décisions thérapeutiques et la possibilité d'un retour au domicile (RAD) dépendent de multiples facteurs dont la pertinence a peu été explorée.

Après un premier épisode de CC, une récidive intervient le mois suivant dans 20% des cas [127], et jusqu'à 50% dans les 3 ans [128](70% après la seconde crise [129]). Ces données et les facteurs qui y sont associés sont pris en compte pour évaluer l'intérêt de la mise en place d'un traitement anti-épileptique afin de limiter le risque de récurrence. En médecine d'urgence, l'évaluation du risque de récidive et de complication à court terme est cruciale pour décider d'un éventuel RAD. De précédentes études ont retrouvé un taux de récidive convulsive de 18% à 24H d'un passage aux urgences pour convulsion [122,130], 30% en cas d'imprégnation alcoolique.

Les facteurs associés à un risque de récidive à long terme ont été étudiés dans une grande étude multicentrique européenne : the MRC Multicentre trial for Early

Epilepsy and Single Seizures (MESS), laquelle évaluait l'intérêt de l'introduction d'un traitement anti-épileptique immédiat [129]. Dans une étude secondaire, les auteurs ont développé un modèle qui prédit le risque de récurrence à long terme : en fonction du nombre de crises avant la consultation, leurs types, la présence d'une anomalie à l'examen neurologique, ou d'une anomalie électroencéphalographique, les patients étaient considérés comme étant à faible, modéré ou haut risque de récidive à 1, 3 et 5 ans [131]. Toutefois, Kho et coll. rapportaient des résultats différents : les nombre et type de crise ne seraient pas des facteurs associés à des risques de récidive différents. Le seul facteur indépendant retrouvé était le caractère « symptomatique » de la crise (ou encore appelé crise « provoquée », causée par une aggression systémique ou neurologique aigüe comme un traumatisme crânien, l'hypoglycémie, etc.) , comparé aux crises idiopathique (Odds Ratio OR=2.2) [132]. En revanche, très peu d'études ont essayés de relier des paramètres cliniques et biologiques au risque de récidive précoce. A notre connaissance, seule une étude observationnelle a évalué les facteurs associés : la prise d'alcool (OR=1.3), l'hypoglycémie (OR=1.7) et un score de Glasgow (GCS) inférieur à 15 (OR=1.9) ont été décrit comme facteurs de risque indépendant de récidive à 48H [130].

La S100B est considérée comme un marqueur biologique objectif de lésion cérébrale. Son intérêt dans la prédiction de l'état neurologique après arrêt cardiaque ou la gravité des traumatismes crâniens a été largement rapporté. Comme nous l'avons vu précédemment, la combinaison de la copeptine avec des marqueurs spécifiques d'organe comme la Troponine ou le nt-pro BNP peut avoir une grande valeur ajoutée diagnostique ou pronostique. A notre connaissance, aucun de ces 2

biomarqueurs (copeptine et S100B) n'a jamais été évalué dans la prise en charge des crises convulsives et de l'épilepsie.

Nous testons l'hypothèse qu'une approche multi-marqueur incluant un marqueur spécifique (la S100B) et un marqueur généraliste (la copeptine) peut améliorer la prédiction de récidive ou d'aggravation après un épisode convulsif. L'élévation de l'un d'eux pourrait être associée à un risque de complication.

B. Discussion

L'étude internationale de cohorte BISTRO nous apporte deux informations importantes pour l'évaluation des convulsions aux urgences. D'une part, nous avons mis en évidence quatre critères cliniques, indépendamment associés au risque d'aggravation ou de récidive. L'âge, le caractère provoqué de la crise, une première crise ou une crise partielle complexe sont des critères de gravité à prendre en compte dans l'évaluation du risque précoce d'aggravation, et qui peuvent aider à guider la prise en charge. Ces résultats comblent un manque dans la littérature et une attente, car les facteurs de gravité à court terme ont été peu étudiés, et étaient nécessaire dans l'établissement de recommandations pour la prise en charge des convulsions aux urgences [133].

D'autre part, nous rapportons que ni la copeptine, ni la S100B ne semble présenter d'intérêt dans l'évaluation initiale après une crise convulsive. Les courbes ROC de ces deux marqueurs pour la prédiction du critère principal montrent un faible pouvoir discriminant, avec une AUC inférieure à 0.6. Aussi, comme on le voit sur le tableau 2, même en faisant varier le seuil, on ne peut obtenir de caractéristiques diagnostiques intéressantes. Malgré un seuil élevé (0.5µg/l), par exemple pour optimiser la spécificité de la S100B (99% [97% - 100%]), la valeur prédictive positive (VPP) reste insuffisante : 87%, avec un large intervalle de confiance [60% - 98%].

Associer deux biomarqueurs aurait pu théoriquement permettre d'améliorer ces performances. Mais la combinaison de la S100B avec la copeptine ne permet toujours pas d'obtenir des résultats satisfaisants. En faisant varier les seuils, on parvient à augmenter la spécificité mais au prix d'une sensibilité rapidement

décroissante. Ainsi, pour les seuils de S100B et copeptine à 0.1µg/l et 100pmol/l respectivement, on trouve une spécificité à 95% [92 – 98%] et une VPP 73% [57 – 86%]. De même, avec des seuils à 0.5µg/l et 100pmol/l : la spécificité à 100% [98% - 100%] est assortie d'une sensibilité à 5%, rendant le test non contributif.

D'autres combinaisons (différents seuils, l'un ou l'autre positif…), ne parviendront pas à donner de caractéristique utilisable en pratique clinique. La S100B et la copeptine ne permettent pas d'aider à la prédiction de l'aggravation (telle que définie dans notre critère de jugement composite), seuls ou en association.

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RESEARCH ARTICLE

Predictive Value of S100-B and Copeptin for Outcomes following Seizure: The BISTRO International Cohort Study

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Abstract

Objective

To evaluate the performance of S100-B protein and copeptin, in addition to clinical variables, in predicting outcomes of patients attending the emergency department (ED) following a seizure.

Methods

We prospectively included adult patients presented with an acute seizure, in four EDs in France and the United Kingdom. Participants were followed up for 28 days. The primary endpoint was a composite of seizure recurrence, all-cause mortality, hospitalization or rehospitalisation, or return visit in the ED within seven days.

Results

Among the 389 participants included in the analysis, 156 (40%) experienced the primary endpoint within seven days and 195 (54%) at 28 days. Mean levels of both S100-B (0.11 μg/l [95% CI 0.07–0.20] vs 0.09 μg/l [0.07–0.14]) and copeptin (23 pmol/l [9–104] vs 17 pmol/l [8–43]) were higher in participants meeting the primary endpoint. However, both biomarkers were poorly predictive of the primary outcome with a respective area under the receiving operator characteristic curve of 0.57 [0.51–0.64] and 0.59 [0.54–0.64]. Multivariable logistic regression analysis identified higher age (odds ratio [OR] 1.3 per decade [1.1–1.5]), provoked seizure (OR 4.93 [2.5–9.8]), complex partial seizure (OR 4.09 [1.8–9.1]) and first seizure (OR 1.83 [1.1–3.0]) as independent predictors of the primary outcome. A second regression

Funding: Roche Diagnostics France (Meylan, France) provided free reagents and kits for S100B assays, and Thermo Fisher scientific B.R.A.H.M.S (Aktiengesellschaft, Hennigsdorf, Germany) provided free reagents and kits for copeptin assays. ThermoFisher BRAHMS provided the copeptin assays free of charge, and funded our biochemistry technicians' time of work in order to process all the analysis. Roche diagnostic provided the S100B assays free of charge. The corresponding author had full access to all the data in the study and final responsibility for the decision to submit for publication. Yonathan Freund received a grant from the French society of emergency medicine (SFMU) and from the Assistance Publique—Hôpitaux de Paris for this study. The funders had no role in the study design, data collection, analysis, and interpretation, or the writing of the report.

Competing Interests: Roche Diagnostics France (Meylan, France) provided free reagents and kits for S100B assays, and Thermo Fisher scientific B.R.A.H.M.S (Aktiengesellschaft, Hennigsdorf, Germany) provided free reagents and kits for copeptin assays. Roche diagnostics and Thermofisher scientific did not participate in the analysis of the data and did not see the manuscript before submission. ThermoFisher BRAHMS provided the copeptin assays free of charge, and funded our biochemistry technicians' time of work in order to process all the analysis. Roche diagnostic provided the S100B assays free of charge. YF, BB, JB, NB, SL, TH, VN and MB had no other conflict of interest with this study. PH received lecture fees and clinical studies fees from Thermofisher scientific and honorarium from ROCHE diagnostic. BR received lecture fees from Thermofisher scientific This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

analysis including the biomarkers showed no additional predictive benefit (S100-B OR 3.89 [0.80–18.9] copeptin OR 1 [1.00–1.00]).

Conclusion

The plasma biomarkers S100-B and copeptin did not improve prediction of poor outcome following seizure. Higher age, a first seizure, a provoked seizure and a partial complex seizure are independently associated with adverse outcomes.

Introduction

Patients attending the emergency department (ED) with seizure account for 0.5 to 7% of all ED visits, and approximately one million visits per year in the United States [1–5]. The impact of one or more seizures on an individual includes the potential for physical trauma, time off work, degeneration into status epilepticus and the risk of a life threatening acute anoxic event [6–8]. Therefore the ability to risk assess for recurrence is of critical importance.

The rate of long term recurrence is high, with a three year risk of 30% after acute symptomatic seizures and 50 to 70% after an unprovoked seizure [9–12]. The rate of early seizure recurrence (ESR) is less well established. ESR rates have been reported to be 19% in the first 24 hours, and up to 30% in cases of alcohol related seizure [4,13]. One prospective study has evaluated predictors of ESR, and found that alcoholism, low plasma glucose, and a Glasgow coma scale (GCS) less than 15 were independently associated with a higher risk of ESR [13]. As the risk of other adverse events, such as hospitalisation or death, following a seizure have not been studied, there may be further variables in addition to the three identified that can assist in the risk stratification of patients presenting to the ED with seizure.

The astroglial S100-B protein is a specific marker of cerebral injury. Raised S100-B has value in predicting adverse neurological outcomes in cardiac arrest and traumatic brain injury [14–16]. S100-B concentration is normal following febrile seizure in children. That febrile seizures are considered to be relatively harmless contributes to the hypothesis that elevated S100-B might predict adverse neurological outcomes [17,18]. Copeptin, the c-terminal part of the vasopressin molecule, is a biomarker of endogenous stress. Recently, it has been described as a good prognostic marker in neurological disorders, such as traumatic brain injury [19], intracerebral hemorrhage, and stroke [20,21].

We hypothesised that these two biomarkers may have an incremental added prognostic value to routine clinical data to predict adverse events following seizure related ED visits.

Methods

Study design, setting and participants

The Biomarkers In Seizure To predict Recurrences and severe Outcomes (BISTRO) is a prospective international cohort study (NCT01774500), conducted from January 2013 to December 2013. The primary objective is to establish the incremental value of combining S100-B and copeptin levels with standard clinical variables to identify patients most at risk of complications following presentation in the ED with seizure.

We enrolled patients from four centres: one in London, UK and three in Paris, France. Participants' informed signed consent was sought prior to enrolment, and institutional review boards from both countries approved the study (Comité de protection des personnes—Paris

Ile de France 6, Paris, France; and NHS Health Research Authority, National Research Ethics Service Camberwell St Giles, United Kingdom). In cases in which informed consent could not be obtained from the patient due to a decreased level of conscious, a next-of-kin signed informed consent was mandatory prior to enrolment. After the patient returned to a normal level of consciousness, their signed informed consent was then sought. When this was not obtained, the patient was excluded from the study.

The study design and report is in accordance with the STROBE statement [22]. Patients were eligible to become study participants if they were 18 years or older and had had one or more convulsive seizures within 24 hours. Patients were excluded if they were less than 18 years; pregnant; prisoners; and those for whom seven or 28 day follow up was deemed impossible. Patients were screened in real time in the EDs of the participating centres.

Outcomes

The primary endpoint was a composite endpoint of seizure recurrence, or all cause death, hospitalisation, or rehospitalisation or return visit in the ED within seven days.

Secondary endpoints included seizure recurrence at seven and 28 days; ICU admission; death within seven and 28 days; and length of hospitalization within seven and 28 days. The decision to hospitalise a patient depends on individual physicians and as such may be considered subjective. To reduce the effect of this subjectivity, a sensitivity analysis was run with a modified primary endpoint that excluded those patients that were hospitalised for less than 24 hours. Finally, as predicting adverse events in discharged patients is of great importance, we ran a sub-analysis focusing only on patients that were not admitted after their first ED visit.

Variables

Clinical and physiological data were recorded; white cell count, sodium, calcium, glucose, and lactate were routinely measured within the participating centres. Venous blood samples were taken in heparinised tubes to measure S100-B and copeptin. The sample for S100-B and copeptin was frozen at -80°C and all samples were measured in a single batch at the end of the study to avoid bias from assay discrepancy. The assay for copeptin measurement was performed on a KRYPTOR analyzer using the commercial sandwich immunoluminometric assay (B.R.A.H.M.S Aktiengesellschaft, Hennigsdorf, Germany). The lower detection limit is 4·8 pmol/L, and the functional assay sensitivity is $<$ 12 pmol/L. The limit of quantification (10% coefficient of variation [CV]) is 14 1 pmol/L. In our laboratory, the CV were found to be $<$5% (4 4% at 28 86 pmol/L and 4·6% at 95·84 pmol/L). S100-B measurement was performed on an Elecsys (Roche Diagnostics, Mannheim, Germany). The lower detection limit is 0·005 μg/L and the functional assay sensitivity is 39 μg/L. In our laboratory, the CV was found to be $<$5%. Copeptin and S100-B determinations were performed blinded to the clinical assessment of the emergency physicians. Follow up was performed either by telephone or hospital visit.

Since the definition of "epilepsy" is controversial, and has varied in recent years [23–25], a patient was considered epileptic if a neurologist had ever diagnosed the condition, if the patient had an unprovoked seizure and evidence of remote CNS lesion or if the patient was currently on antiepileptic drug. A remote lesion is a CNS lesion that is stable and is not acute (for instance a stroke sequellum).This approach is in accordance with recommendations from the International League Against Epilepsy (ILAE) for a pragmatic definition of epilepsy [25]. A seizure was classified, according to ILAE guidelines, as provoked if it could have been related to an acute systemic insult or acute CNS lesion (there are many causes for a provoked seizure, for instance alcohol intoxication, alcohol withdrawal, hypoglycemia) occurring within the previous seven days, or unprovoked if not. Unprovoked seizures were classified as idiopathic, or

remote symptomatic in the presence of a known CNS lesion. Seizures in the setting of sleep deprivation were not considered provoked [26].

Patients were followed up for 28 days, and were called (or visited if still in the hospital) at day seven and 28 to assess endpoints. Participants with missing data regarding the two biomarkers, and participants lost to follow up were excluded.

Study size

On the basis of pre-existing literature, we estimated the rate of the primary endpoint at day seven to be 20%. To avoid overfitting and in order to be able to include at least 10 variables in the logistic regression model, there needed to be at least 100 events in our sample [27]. Furthermore, this minimal number of 100 events is warranted for external validation [28]. Therefore a total sample size of 500 participants was required for this study. An interim analysis of outcome showed a higher rate of the endpoint than expected (35%), which reduced the required sample size to 350 participants.

Statistical analysis

Data are expressed as mean ± standard deviation (SD) for Gaussian variables; median and 25 to 75% interquartile range for non-Gaussian variables; and number and percentage for categorical variables with 95% confident interval. Normality was assessed using the Kolmogorov-Smirnov test. Measures of diagnostic accuracy were calculated with their 95% confidence intervals (CI) for S100-B and copeptin. Receiving operator characteristics (ROC) curves were constructed and their area under the curve was calculated. Thresholds were determined using the Youden's method. Comparison of the two groups was performed using the Student t test, the Mann-Whitney U test, and Fisher's exact method when appropriate.

A multiple logistic regression was performed to assess independent variables associated with the primary endpoint, and odds ratios (ORs) with their 95% CI were calculated. To avoid overestimation, a conservative approach was used and all clinically relevant variables were included [29]. These variables were determined a priori upon previous literature and clinical relevance (namely age, first seizure, history of epilepsy, neuromuscular impairment, chronic alcohol intake, focal neurological deficit, complex partial seizure, provoked seizure, GCS < 15, body temperature $> 37{\cdot}5$°C) and the two studied biomarkers, S-100 and copeptin. Correlation between all variables were calculated, and in case of a coefficient of correlation $R^2 > 0{\cdot}6$, only the most clinically significant variable was entered in the model. Calibration of the model was estimated with Hosmer-Lemeshow test, and discrimination with the c-index. Internal validation was assessed using the bootstrap resampling method (n = 500, without replacement) [30]. To present the internal validation,the difference (optimism) between the c-statistics observed in the population and in the bootstrapped sample was calculated [30].

All analyses were performed using SPSS software (IBM, Armonk, NY), all comparisons were two-tailed and a p value of 0·05 was required to reject the null hypothesis. The statistical plan was decided before the onset of the study.

Results

In the period of inclusion, 443 participants were enrolled. Twenty two participants had no S100-B and copeptin measurements, and 32 were lost to follow up (Fig 1). Therefore 389 participants were included in the analysis, of which 87 (22%) were from the United Kingdom and 302 (78%) from France. The mean age of the studied population was 44 years (SD 18), and 58% were male. One hundred and thirty (33%) presented to the ED with a first seizure and 259

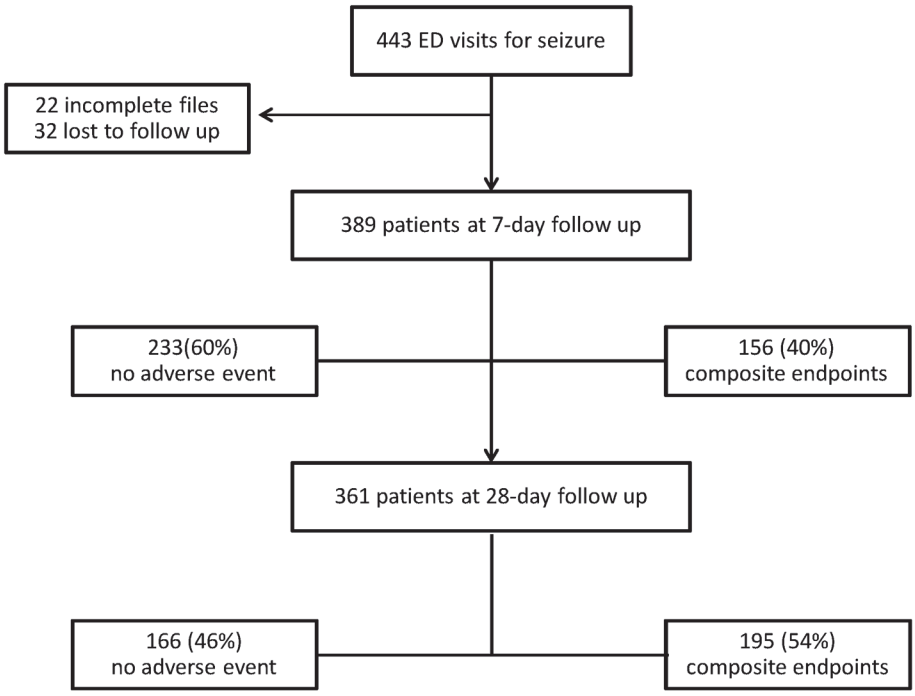

Fig 1. Flow chart ED: emergency department. Composite endpoint of recurrence, hospitalization or death at day seven.

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(67%) were considered epileptic according to the definition above. Main baseline characteristics are summarized in Table 1.

One hundred and fifty six participants (40%) experienced the primary endpoint of death, hospitalization, seizure recurrence, rehospitalisation or return visit to the ED within seven days and 195 (54%) at 28 days. The primary endpoint occurred in 56%, 40%, 31% and 26% in participants from Royal London Hospital, Pitié-Salpêtrière, Tenon and Lariboisière hospitals respectively (p = 0·003 for UK vs France). Sixty patients (15%) had a seizure recurrence within seven days. Main outcomes are summarized in Table 2.

Copeptin and S100-B were significantly higher in participants that experienced the primary combined endpoint than in the others: 0·11 [0·07–0·20] vs 0 09 [0 07–0 14] µg/l (p = 0·02) for S100-B and 23 [9–104] vs 17 [8–43] pmol/l (p<0 001) for copeptin (Fig 2).

ROC curves for S100-B and copeptin are reported in Fig 3, with a respective area under the curve of 0 57 [95% CI 0·51–0·64] and 0·59 [95% CI 0·54–0·64] (p<0·05 for both). Using Youden's method, a threshold value of 0 1 µg/l for S100-B and 100 pmol/l for copeptin was found, which corresponded to a sensitivity and specificity of 57% [49–65%] and 53% [46–59%] respectively for S100-B, and 24% [18–31%] and 92% [88–95%] respectively for copeptin. Complete diagnostic performances are reported in Table 3 with different thresholds. Of note, we studied "positive S100 AND positive copeptin", as well as "Positive S100 OR positive copeptin", with different thresholds and we found that no combination resulted in satisfactory diagnostic performances (data not shown). When considering more homogenous populations, for example epileptic patients, or patients with provoked seizure, neither of these two biomarkers showed good diagnostic performances (data not shown).

A multivariable logistic regression was performed with pre-specified variables. "Epilepsy" as a variable was not included because it was correlated with the variable "first seizure" (R^2 = 0.66). We kept "first seizure" instead of "epilepsy" in the model, because the diagnosis of

Table 1. Characteristics of study cohort.

		Total	All patients		No event in 7 days		Recurrence or severe outcome at day 7	
		389	389		233	(60%)	156	(40%)
Characteristic	Age, mean (SD), y		44	(18)	40	(16)	51	(20)
	Sex Male, No. (%)		229	(58%)	143	(61%)	86	(55%)
	Sex Female, No. (%)		160	(42%)	90	(39%)	70	(45%)
	Seizure in the ED, No. (%)		73	(19%)	21	(9%)	52	(33%)
	Seizure		259	(67%)	170	(0.72)	89	(57%)
Past Medical History, No. (%)	Epilepsy		217	(56%)	147	(63%)	70	(45%)
	Stroke		32	(8%)	16	(7%)	16	(10%)
	Meningitis		14	(4%)	7	(3%)	7	(4.5%)
	Neuromuscular impairment		22	(6%)	7	(3%)	15	(10%)
	Chronic alcohol intake		50	(13%)	20	(9%)	30	(20%)
	Drug		13	(3%)	8	(3%)	5	(3%)
	Benzodiazepin		56	(14%)	37	(16%)	19	(12%)
Current medication, No. (%)	Anti epileptic drug		172	(44%)	114	(49%)	58	(37%)
	Headache		100	(26%)	62	(27%)	38	(24%)
On Examination, No. (%)	Photophobia		13	(3%)	9	(4%)	4	(3%)
	Confusion		43	(11%)	14	(6%)	29	(19%)
	Neurological deficit		13	(3%)	1	(0.5%)	12	(8%)
	Partial simple		24	(6%)	13	(6%)	11	(7%)
Type of seizure, No. (%)	Complex partial		41	(10%)	13	(6%)	28	(18%)
	Generalised tonic clonic		290	(75%)	179	(77%)	111	(71%)
	Absence		31	(8%)	22	(9%)	9	(6%)
	Acute Symptomatic		67	(17%)	17	(7%)	50	(32%)
	Remote symptomatic		49	(13%)	25	(11%)	24	(15%)
	Idiopathic		273	(70%)	191	(82%)	82	(53%)
	Witnessed		280	(72%)	160	(69%)	120	(77%)
	Time from Seizure to ED visit, median [IQR], hours		1.5	[1–2]	1.5	[1–2]	0.7	[0–2]
	Heart rate, mean (SD)	378	89	(19)	89	(19)	90	(17)
Physiological parameters on admission	Systolic BP, mean (SD)	380	129	(21)	129	(19)	129	(24)
	Diastolic BP, mean (SD)	380	77	(15)	77	(13)	79	(17)
	Temperature, mean (SD)	376	36.6	(0.6)	36.6	(0.5)	36.8	(0.7)
	GCS, median [IQR]	379	15	[15–15]	15	[15–15]	15	[15–15]
	GCS<15, No (%)	389	45	(12%)	25	(11%)	20	(13%)
	Pulse oxymetry, median [IQR]	380	97	[96–99]	98%	[96–99]	97%	[95–99]
	WBC (Giga/l), median [IQR]	325	9.8	[7.0–13]	9.5	[6.5–12.7]	10.4	[7.4–13]
Laboratory results	Glucose (mmol/l), median [IQR]	270	6.1	[5.2–7.3]	5.8	[5.1–6.8]	6.4	[5.4–8]
	Sodium (mmol/l), mean (SD)	365	137	(12)	137	(13)	137	(11)
	Calcium (mmol/l), median [IQR]	289	2.3	[1.3–2.4]	2.4	[2.3–2.5]	2.3	[1.2–2.4]
	Lactate (mmol/l), median [IQR]	176	1.9	[1.2–3.6]	1.65	[1.2–3.3]	2.1	[1.3–3.7]
	S100B (µg/l), median [IQR]	389	0.10	[0.07–0.16]	0.09	[0.07–0.14]	0.11	[0.07–0.2]
	Copeptin (pmol/l), median [IQR]	389	19	[8–54]	17	[8–43]	23	[9–104]

SD, standard deviation; IQR, 25–75% interquartile range; ED, emergency department; GCS, Glasgow coma scale; WBC, white blood cells. All laboratory results were obtained from venous blood.

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Table 2. Outcomes and follow up of the study cohort.

		Total	All patients	
Disposition from ED	Home	**389**	243	(63%)
	Observation unit		95	(29%)
	Hospitalisation		126	(32%)
	Admission in ICU		11	(3%)
	Admission in neurosurgery		15	(4%)
	Death		2	(1%)
Follow up day 7	Seven days free of hospital	**389**	224	(58%)
	Recurrence		60	(15%)
	Re hospitalisation		16	(4%)
	Number of hospital free days, median [IQR]		7	[4–7]
	ICU admission		14	(4%)
	Death		5	(1%)
Follow up day 28	28 days free of hospital	**361**	185	(51%)
	Recurrence		97	(27%)
	Rehospitalisation		29	(8%)
	Hospital free days, median [IQR]		28	[25–28]
	ICU admission		16	(4%)
	Death		10	(2%)

ED, emergency department, ICU, intensive care unit; IQR, 25–75% interquartile range.

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epilepsy can be more subject to diagnostic disagreement than a "first seizure". Two models are presented; one not including and the other including the biomarkers. In the first model independent risk factors for the primary outcome were found to be higher age; complex partial seizure; provoked seizure; and first seizure (Table 4). Discrimination of the model was good with a c-statistic of 0 77 [95% CI 0·72 to 0·81] and Hosmer-Lemeshow goodness-of-fit test had a p = 0·51. Bootstrap sampling confirmed the internal validity of the model, with an optimism of

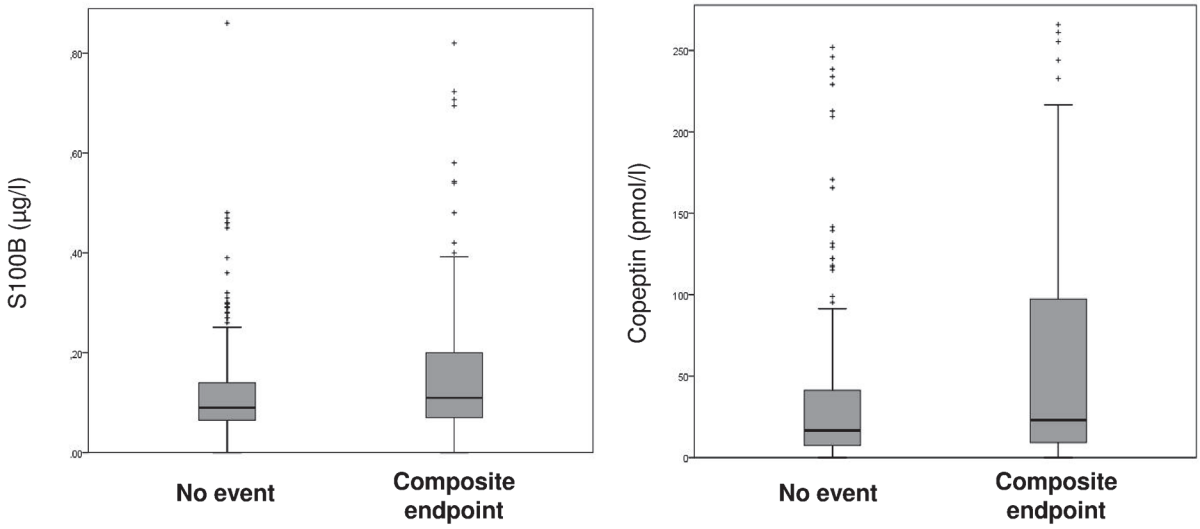

Fig 2. S100B and copeptin values in the two groups. Box plot with median, interquartile range, and 5th and 95th centile. Composite endpoint of recurrence, hospitalization or death at day seven.

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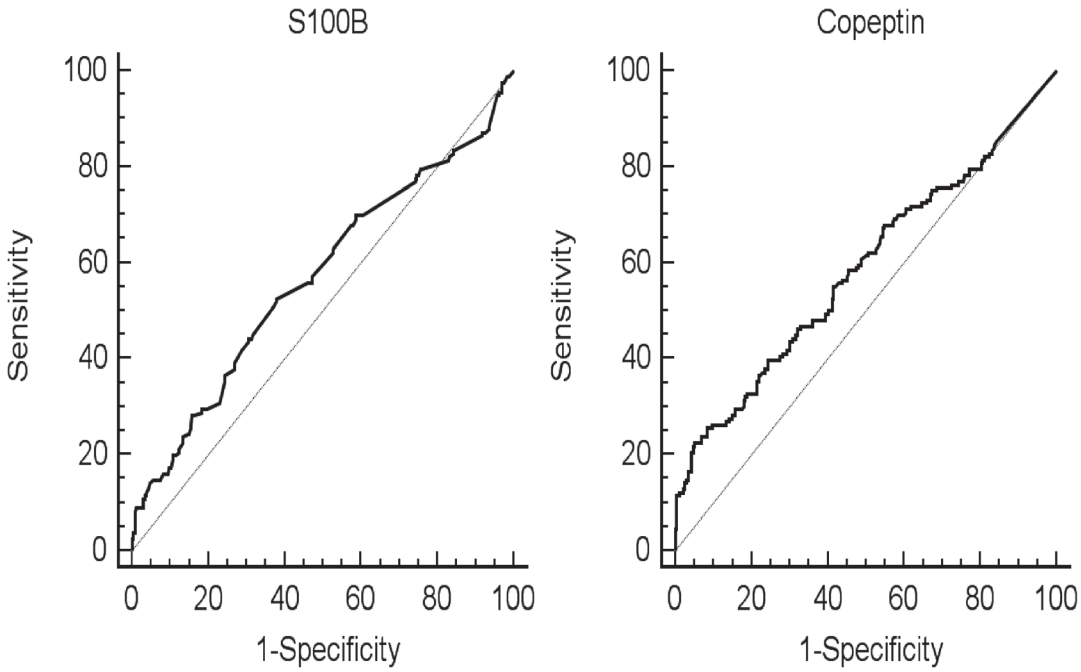

Fig 3. Receiving operator characteristics curve for Copeptin and S100B. Area under the curve 0.57 [95% CI 0.51–0.64] for S100B, p = 0.01, and 0.59 [95% CI 0.54–0.64] for copeptin, p = 0.02.

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0.01, and a corrected c-stat of 0.76 When adding S100-B and copeptin, the model was left unchanged, and neither of the two biomarkers was independently associated with the primary endpoint (c-stat 0.78, optimism 0.02, corrected c-stat 0.76).

With a modified primary endpoint that excluded those with hospitalisation for less than 24 hours, there was no improvement in terms of diagnostic performances for either of the two biomarkers. The clinical model of logistic regression showed one supplemental variable independently associated with the endpoint: pre-existing neuromuscular impairment (OR 11·9 [95% CI 1·44–98·60]).

Finally, the subgroup of participants that were not admitted following their ED visit was analysed. There were 263 participants (69%) that were discharged home from the ED. Amongst them, 30 (11%) met the primary endpoint within seven days. Values of S100-B and copeptin were similar in the two groups, with a median of respectively 0·09 μg/l and 17 pmol/l. Complex partial seizures was the only significant predictor of increased risk of recurrence (OR 5·7

Table 3. Diagnostic performances of S100-B and Copeptin, and 95% confidence interval.

Biomarker	Threshold	Sensitivity		Specificity		PPV		NPV		LR+		LR-	
S100-B	0.1	57%	[49%- 65%]	53%	[46%- 59%]	45%	[38% -52%]	65%	[57%- 71%]	1.21	[0.99–1.46]	0.81	[0.65–1.01]
(μg/l)	0.2	24%	[18%- 32%]	85%	[80%- 89%]	52%	[40%- 64%]	63%	[57%- 68%]	1.62	[1.07–2.46]	0.89	[0.80–0.98]
	0.5	8%	[5%- 14%]	99%	[97%- 100%]	87%	[59%- 98%]	62%	[57%- 67%]	9.71	[2.6–58]	0.92	[0.87–0.96]
Copeptin	14	67%	[59%- 74%]	45%	[39%- 52%]	45%	[39%- 52%]	67%	[59%- 74%]	1.22	[1.04–1.44]	0.73	[0.56–0.95]
(pmol/l)	50	33%	[26%- 40%]	79%	[73%- 83%]	51%	[40%- 61%]	64%	[58%- 69%]	1.52	[1.09–2.13]	0.86	[0.75–0.97]
	100	24%	[18%- 31%]	92%	[88%-95%]	66%	[52%- 78%]	64%	[58%- 69%]	2.91	[1.76–4.96]	0.83	[0.75–0.91]

PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR-, negative LR.

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Table 4. Adjusted odds ratios of independent predictors for composite endpoint.

Variables	Adjusted ORs	95% CI	Variables	Adjusted ORs	95% CI
Provoked seizure	4.93	2.47–9.84	Provoked seizure	4.71	2.32–9.56
Complex partial	4.09	1.84–9.08	Complex partial	4.26	1.90–9.52
First seizure	1.83	1.10–3.02	First seizure	1.73	1.03–2.89
Age (per 10 year older)	1.27	1.11–1.45	Age (per 10 year older)	1.26	1.11–1.45
			S100B	3.89	0.80–18.9
			Copeptin	1.00	1.00–1.00

OR, odds ratio; CI, confident interval.

a) clinical model, Hosmer-Lemeshow goodness-of-fit statistics p value 0.5, c-stat 0.77.

b) model with S100-B and copeptin, Hosmer Lemeshow goodness-of-fit statistics p value 0.04, c-stat 0.78.

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[95% CI 1·96–16·7]). No association was found between the level of S100-B or copeptin and the rate of secondary endpoints—only copeptin was associated with ICU admission at day seven and 28 (Table 5).

Discussion

With this study, we aimed to determine whether S100-B and copeptin are of added prognostic value to usual assessment following seizure. The first result from our study is a negative result: measurement of S100-B and copeptin has no significant added value to predict the risk of seizure recurrence or severe outcome. We found that the primary endpoint was more frequent than we expected with a rate of 40%. Finally, we present four independent clinical factors that are associated with a significant increased risk of adverse events after a seizure: higher age; acute symptomatic seizure; complex partial seizures; and a first seizure.

Although the long term rate of recurrence is well known, there is scarce data on the risk of early seizure recurrence. In its last clinical policy on evaluation of adults presenting with seizures, the American College of Emergency Physicians [31] tried to identify patient that do not need to be admitted to prevent adverse events. In contrast with literature regarding long term outcome, their level C recommendations lack studies focusing on early recurrence. As stated by Huff et al., the immediate need for admission and observation after ED evaluation has not been specifically addressed [31]. We chose a composite endpoint of early complications after ED visit that included seizure recurrence; hospital admission; death within seven days; or return visit to hospital within seven days. We consider these endpoints to be sufficiently severe that they merited being addressed collectively. The timeframe of seven days is consistent with previous literature [32,33].

Table 5. Median of S100B and copeptin, with their 25%-75% interquartile range.

		Day seven		Day seven		Day 28		Day 28	
		No recurrence	Recurrence	No ICU admission	ICU admission	No recurrence	Recurrence	No ICU admission	ICU admission
S100B	(μg/l)	0.1 [0.07–0.16]	0.09 [0.06–0.17]	0.1 [0.07–0.16]	0.1 [0.08–0.20]	0.1 [0.07–0.18]	0.09 [0.06–0.15]	0.09 [0.06–0.16]	0.11 (0.08–0.20]
Copeptin	(pmol/l)	19 [8.3–54]	18 [5.2–48.5]	19 [8–53]	33 [8.2–296]	23 [9.9–66.2]	17 [0–47]	20 [8.6–54]	74 [11.1–311]

ICU: Intensive Care Unit.

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In recent years S100-B has been reported to have a very high specificity for death (95% to 98%) and unfavourable neurological outcomes (85 to 98%) [34], and a very high sensitivity for the diagnosis of brain lesions (99 to 100%) [16,35] in traumatic brain injury. In the context of seizure, we report very low diagnostic performances of S100-B, with failure to obtain thresholds that would allow greater sensitivity with acceptable specificity, or vice versa. There was a very high rate of S100-B false positive (47% and 15% for a respective threshold of 0·1 and 0·2 μg/l), i.e. S100-B was raised in many cases that did not meet the primary endpoint. This suggests that there is a pathophysiological increase in blood concentration of S100-B after a seizure, regardless of whether that patient will go on to develop the primary endpoint or not. Similarly, we report no added value of copeptin in the setting of convulsive seizure. We failed to determine a threshold of S100-B or copeptin value that can help the clinician either to rule in or exclude the occurrence of adverse events.

The high frequency of the primary endpoint is in contrast to previously published work. This could be explained by the fact that our endpoint is a composite whereas previous studies report singular primary endpoints such as seizure recurrence. In their study in France, Choquet et al. found an early seizure recurrence rate of 19% (within 24 hours) [13], and Breen et al. suggested that a rate of at least 28% patients that were not initially admitted experienced the endpoint in the next six weeks [36]. In our study, more than a tenth of patients who were initially not admitted had an early seizure recurrence or re hospitalization within seven days.

The four independent factors we found to be associated with a significantly increased risk of adverse events after a seizure were higher age; provoked seizure; complex partial seizures; and first seizure. Besides higher age, those three conditions can contribute to the overall risk assessment a physician makes when encountering a patient that has just had a seizure. Other factors reported in the literature as carrying an increased risk of recurrence are a higher blood glucose level, a decreased GCS, and a context of alcoholism. We confirmed the influence of blood glucose level although only in the univariate analysis. However, we did not find that a decreased GCS was associated with the occurrence of adverse events—probably due to a lack of power. We also found that provoked seizure (therefore including those in the context of alcohol) is an independent risk factor of recurrence and severe outcome. This is a very valuable result as most previous studies focused on the risk of long term recurrences, and reported that provoked seizures have a lower rate of recurrence at three years (30 vs 50–70% [9–12,26]).

Limitations

Our study has some limitations. There is a significant difference in the rate of the endpoint between France and UK. There may be inclusion bias as the ED systems of the two countries are markedly different: in the UK centre, less severe patients were managed in a different part of the ED (out-of-hours general practitioners' clinic, or minors unit) where recruitment did not take place. Another limitation was the choice of our composite endpoint that included subjective data such as "hospitalization". However, we determined that inclusion of hospitalization was not a serious shortcoming by running a sensitivity analysis with modified composite endpoints (with the exclusion of patients hospitalized less than 24 hours for example, and focusing only on critically ill patients), and the conclusions remained the same. Finally, there may be an element of inclusion bias because the diagnosis of seizure may be uncertain in the ED, and consequently we may have included some patients that did not have a true epileptic seizure, and may have had a pseudo-epileptic seizure or convulsive syncope. This limitation is inherent to the design and reflects the day to day work of an emergency physician, in which it is sometimes impossible to fully confirm than an epileptic seizure has occurred. In the same way, the collected data on the type of seizure were made upon patient and witness interrogation, and are

consequently subject to bias. This again mirrors the real life information to which a clinician has access. A third of patients had no witness account of their seizure. To avoid inconsistencies in classification of seizure type, we classified any seizure with loss of consciousness as generalised although some of them could have been absence or focal seizure with lost of consciousness.

Conclusion

In summary, S100-B and copeptin have very low added value to predict adverse events after an ED visit for seizure. We report four independent clinical predictors of early seizure recurrence and severe outcome: higher age; provoked seizure; complex partial seizure; and first seizure. Since the rate of adverse events is high (40%) we suggest that these conditions should alert emergency physicians to increased risk and lower the threshold for admission to the hospital.

Acknowledgments

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Conceived and designed the experiments: YF BB RP BR PH. Performed the experiments: YF BB JB NB SL TH. Analyzed the data: YF VN BR. Contributed reagents/materials/analysis tools: MB. Wrote the paper: YF BB RP BR PH.

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QUATRIEME PARTIE :

Discussion

I) Discussion

Nous avons évalué ici dans trois situations cliniques différentes l'intérêt potentiel du dosage combiné de deux biomarqueurs. Sur le plan théorique, l'association de deux marqueurs non redondants pourrait permettre une meilleure précision pour la confirmation ou l'exclusion d'un diagnostic aux urgences, ou pour évaluer le pronostic et le risque d'aggravation. Le principe sous-jacent à ces trois travaux était donc d'associer un biomarqueur spécifique d'un organe ou d'une pathologie (la troponine pour le SCA, la PCT pour l'infection bactérienne et la S100B pour les lésions cérébrales) avec un biomarqueur non spécifique, généraliste, marqueur de gravité ou reflétant un état pathologique aigu (la copeptine comme marqueur de stress endogène, ou le lactate comme marqueur d'hypoperfusion tissulaire).

A. Dans le sepsis

Nos premiers résultats sont positifs : l'association de la PCT et du lactate est plus précise et plus informative que le dosage singulier de chacun d'entre eux. On a vu sur notre échantillon que l'élévation de l'un ou de l'autre pouvait être complémentaire, et que l'élévation des deux en même temps était associée à une plus forte sévérité de la pathologie. De nombreux biomarqueurs sont régulièrement évalués à la recherche d'un bon candidat pour le diagnostic précoce d'un état septique grave, ou d'un bon marqueur pronostique dans le sepsis. M Levy soulignait qu' « il serait bon de pouvoir identifier le sepsis grave avant qu'il soit évident, mais jusqu'ici, on doit attendre une défaillance d'organe » [ou une hypoperfusion tissulaire] [134]. La PCT, « champion jusqu'ici » dans ce domaine d'après Frank Gu[134], n'a pas trouvé de compétiteur pour l'instant. Tout récemment, la Presepsin (sous-type

soluble du CD14) a montré des résultats prometteurs [135]. Parallèlement au développement et à l'évaluation de ces nouveaux/futurs biomarqueurs, la réponse pourrait se trouver dans l'association de biomarqueurs connus, comme nous l'avons fait avec la PCT et le lactate. De même, Gibot et al. ont montré l'intérêt d'un score qui associe trois biomarqueurs : l'expression du high affinity immunoglobulin-FC fragment receptor CD64 on polymophonuclear (PMN CD64), la PCT et le soluble triggering receptor expressed on myeloid cells-1 (STREM-1). Les auteurs rapportent sur une cohorte de dérivation et une de validation, parmi des patients hospitalisés en réanimation, que l'apport combiné de ces trois marqueurs est meilleur que l'apport individuel de chacun d'entre eux [136]. Sur une cohorte de validation de 300 patients, un bioscore est attribué à chaque malade, correspondant au nombre de biomarqueurs positifs parmi les trois testés. Ainsi, plus le score est élevé, plus il est prédictif de sepsis comme on le voit sur cette figure :

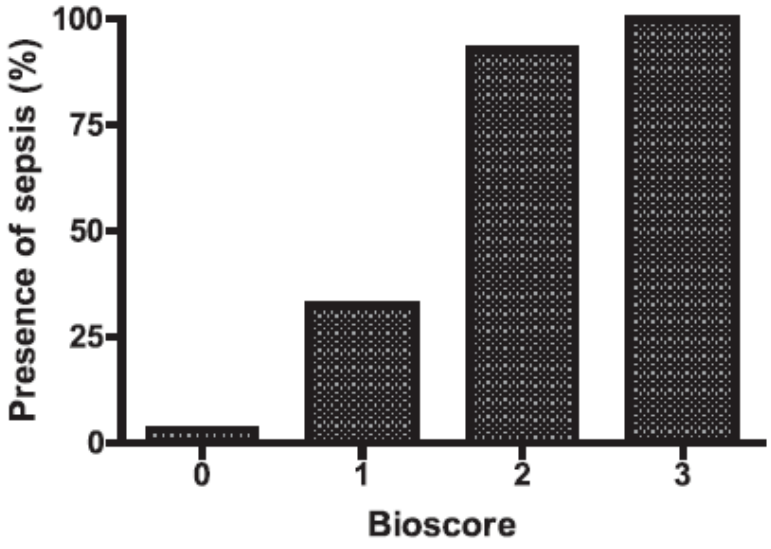

Figure 6 : d'après Gibot et al. [136] Taux de patients avec sepsis en fonction du bioscore.

Les performances diagnostiques de ce score s'avèrent très utiles car l'excellente spécificité du score ne se fait pas au détriment de la sensibilité : 80% des patients peuvent rapidement avoir un diagnostic confirmé ou exclus de sepsis. Nous avons procédé de même sur notre cohorte, et cherché à savoir si le dosage combiné de la PCT et du lactate pouvait améliorer la prédiction d'un « mauvais pronostic », défini comme un critère combiné de mortalité et admission en réanimation. Ces deux variables ressortaient comme critères indépendants dans l'analyse multivariée. Ainsi, en comptant le nombre de biomarqueurs positifs (seuil à 0.80 ng/ml pour la PCT et 2 mmol/l pour le lactate), le risque de « mauvais pronostic » augmente de 12% pour zéro marqueur positif, à 23% pour un marqueur positif, et à 56% pour les deux.

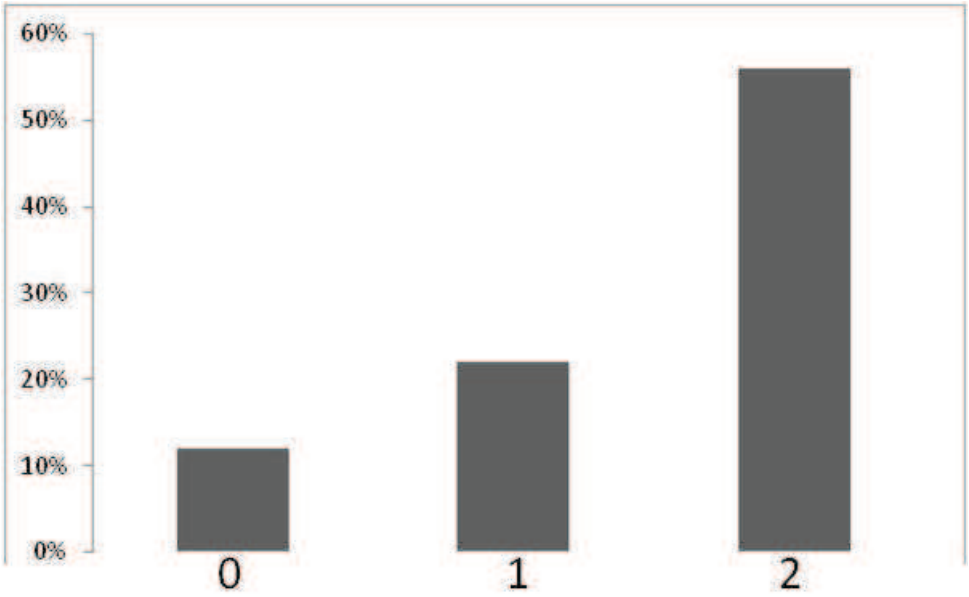

Figure 7 : Risque d'aggravation selon le nombre de marqueurs positifs parmi PCT et lactate. Cohorte Freund et al. PCT/lactate

La positivité de deux biomarqueurs sur les trois testés dans le bioscore de Gibot et al. impliquait un risque de sepsis de plus 90%. De manière similaire dans notre étude, la positivité du lactate et de la PCT identifiait une population avec près de 60% de patients à risque. Les études sur l'approche multimarqueur dans le sepsis sont extrêmement rares, alors que la recherche sur les biomarqueurs dans le sepsis représente une littérature abondante [136–138]. L'étude de Masson et al.[135] sur l'apport respectif de la presepsine et de la PCT montre bien que la valeur intrinsèque de chaque biomarqueur peut être très bien rapportée, mais leur apport combiné n'a pas été évalué.

B. Dans la pathologie cardio-vasculaire

La combinaison de biomarqueur a été plus fréquemment étudiée dans la pathologie cardiovasculaire ou en oncologie, en particulier du fait de l'existence d'un gold standard – la troponine pour le SCA par exemple. Ainsi, lors de l'évaluation d'un nouveau marqueur M, la question n'est pas tant de savoir si ce marqueur a de bonnes qualités diagnostiques, mais surtout de questionner sa valeur ajoutée au biomarqueur de référence. L'évaluation d'un nouveau marqueur dans le cas de pathologies déjà balisées par des marqueurs existants ne devrait se faire qu'en fonction de sa capacité à améliorer leur précision [139]. Ainsi, comme le rapporte Vasan [140], même si la CRP a été montré comme étant un bon marqueur associé au risque vasculaire dans deux études différentes de haut niveau de preuve, elle ne présente pas d'intérêt supplémentaire par rapport aux modèles existant déjà, et sa combinaison au méthodes habituelles de prédiction n'a pas montré un intérêt supplémentaire.

C'est surtout dans le contexte du SCA que l'on retrouve les premières études sur une approche multimarqueur aux urgences. Différents candidats sont donc comparés à la troponine, la référence, et leur valeur ajouté est évaluée. La valeur ajoutée de la myoglogine et des CK-MB à la troponine est par exemple démontré par l'étude CHECKMATE [141], et des scores pronostiques se basant sur le nombre de marqueurs élevés sont construits et validés [142].

Le défi actuel pour le diagnostic du SCA aux urgences est d'identifier un marqueur ou une combinaison qui pourrait exclure ce diagnostic en un seul prélèvement. De nombreuses stratégies alliant plusieurs biomarqueurs et plusieurs prélèvements (cinétique) ont été publiées. Ainsi, on peut écarter le diagnostic de SCA avec une très grande précision après deux dosages de CK/myoglobine/troponine à 90 minutes d'intervalle [116]. Reichlin et al. ont les premiers évalué l'apport de la copeptine à la troponine, et leur première étude suggère dès 2009 que cette association pourrait permettre de limiter la nécessité de surveillance et de dosages sériés d'enzymes cardiaques. Un seul dosage à l'admission de troponine et copeptine avait une VPN de 99,7%. Notre étude confirme ces excellents résultats, et en particulier dans le sous-groupe de patients à faible probabilité clinique pré-test, nous rapportons une VPN à 100%. Ces résultats ont été corroborés par la suite dans plusieurs études interventionnelles de grandes tailles [143]. L'intérêt de cette combinaison a été tout récemment confirmé par une étude d'impact [144]: l'utilisation de la copeptine en association avec la troponine chez les patients à risque faible ou intermédiaire de SCA est sûre, et diminue la durée de passage aux urgences.

Le développement de nouvelles troponines plus sensibles n'a pas suffit à sécuriser la prise en charge aux urgences sur un seul dosage. Nous avons testé de même la valeur ajoutée de la copeptine à la troponine hypersensible : dans cette étude ancillaire (appendice 1), nous retrouvons une sensibilité et une VPN de 100%, quelle que soit le niveau de probabilité pré-test.

C. Autres situations pathologiques aux urgences

L'essor de la S100B dans l'évaluation du traumatisme crânien mineur aux urgences et le succès des études à son propos ont popularisé cette protéine, et en ont fait un bon candidat pour évaluer la gravité des patients après une crise convulsive. Malheureusement, notre étude prospective n'a rapporté qu'une faible association entre la valeur de S100B et l'atteinte neurologique ou le pronostic. Même associée à la copeptine, marqueur à l'inverse sensible et peu spécifique, nous n'avons pu mettre en évidence de seuils qui pourraient guider le clinicien dans la prise en charge des convulsions aux urgences. Cette absence de résultats intéressant réside probablement en partie dans le fait que la S100B n'est en fait pas un marqueur spécifique de la convulsion. D'autres marqueurs potentiellement plus spécifiques seront testés prochainement comme l'Ischemia Modified Albumin [145], la Neurone Specific Enolase [146], ou encore le GFAP. De même, la copeptine, trop sensible, semble s'élever physiologiquement lors d'une crise généralisée même en l'absence de retentissement ou de facteurs de gravité. Cet effet est déjà bien connu sur le lactate, autre marqueur non spécifique d'organe, qui s'élève fréquemment lors d'une crise convulsive [147].

L'approche multimarqueurs aux urgences est aussi actuellement testée dans le contexte de la dyspnée aigue. En 2012, Eurlings et al. ont publié une étude prospective évaluant l'apport pronostique du dosage simultané du Nt pro BNP, la troponine hyper-sensible, la Cystacine-C, la CRP hyper-sensible et la Galectin-3, sur la mortalité à 90 jours chez 603 patients consultant aux urgences avec une dyspnée aiguë. A l'exception de la Galectin-3, ces biomarqueurs étaient indépendamment

associés à un risque accru de mortalité en analyse multivariée, et ont constitué le « panel de biomarqueur » évalué. Ainsi, comme on a pu le voir précédemment dans d'autres études, les auteurs ont construit un score en attribuant un point par biomarqueur dont la mesure dépasse le seuil. Ainsi, chaque point supplémentaire était indépendamment associé à une surmortalité à 90 jours avec un Odds Ratio de 2,95 [IC 95% 2,3 – 3,8].

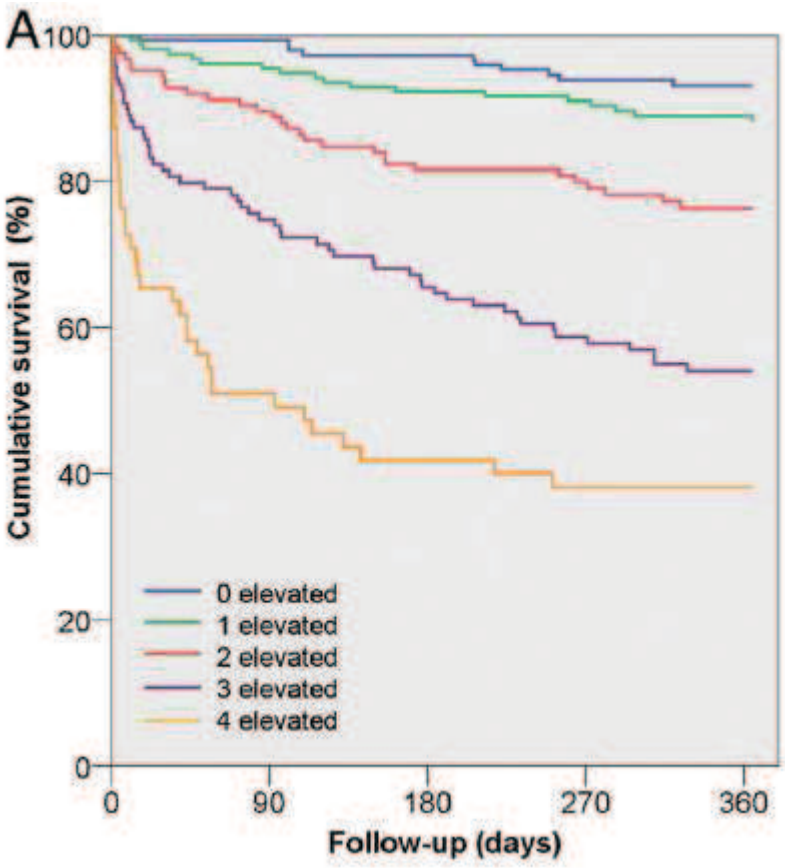

Figure 8 : d'après Eurlings et al. [148]. Courbe de survie (Kaplan-Meier) selon le nombre de marqueurs élevé

De manière intéressante, chaque combinaison de deux ou de trois biomarqueurs était associée à une surmortalité similaire quelle que soit la combinaison. Et l'effet cumulatif persistait après avoir séparé les patients selon l'origine cardiaque ou non

de la dyspnée, et la présence ou non d'une insuffisance rénale comme présenté ci-dessous :

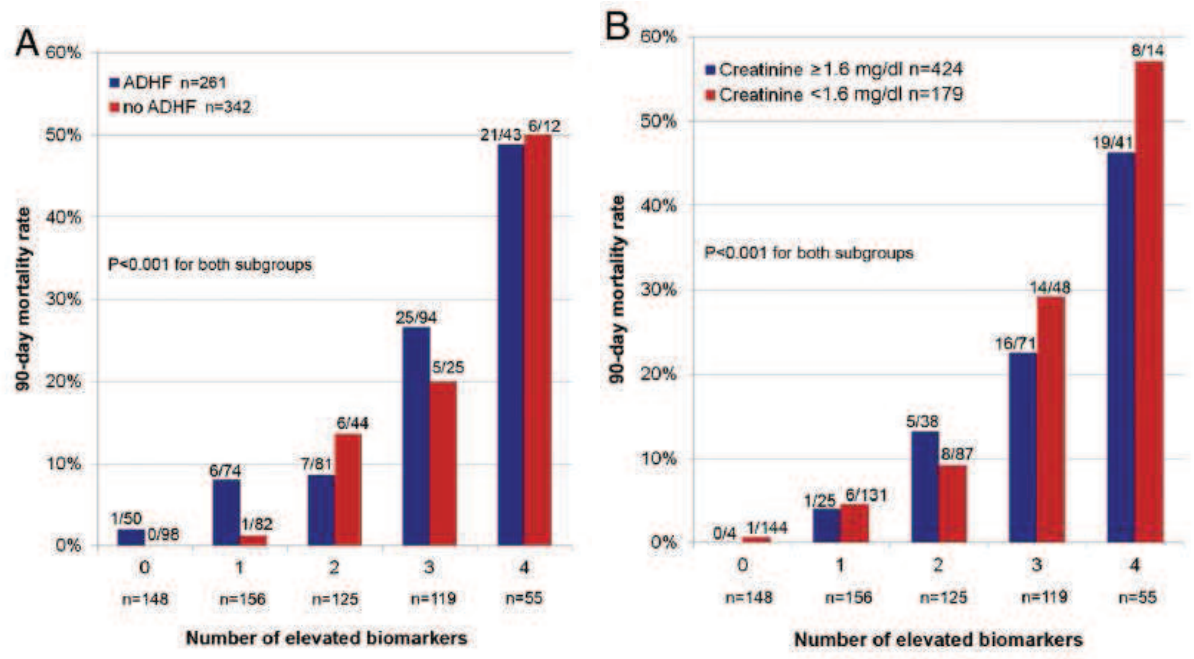

Figure 9 : d'après Eurlings et al. [148] Mortalité à 90 jours selon le nombre de biomarqueurs élevés
stratifié selon la cause cardiaque ou non de la dyspnée (A)
stratifié selon la présence ou non d'une insuffisance rénale (B)

Avec d'autres biomarqueurs, nous avons de même récemment cherché à évaluer dans l'étude prospective multicentrique BIODINER l'intérêt pronostique de la copeptine, la PCT, le MR pro-ANP, MR pro-ADM, et la pro-endothelin dans les.dyspnées aiguës sévères (Appendice 2). Sur un échantillon de 394 patients, 137 (35%) ont été classé comme « évolution défavorable » - un critère combiné regroupant admission en réanimation, nécessité d'oxygénothérapie à haute concentration ou ventilation mécanique, perfusion d'amines vaso-actives, ou décès. Dosés à J0 ou à J1, aucun des cinq biomarqueurs n'a présenté de performances

diagnostiques notables (cf Appendice 2). En outre, l'AUCROC de chacun de ces marqueurs ne dépasse pas 0.61, suggérant un très faible pouvoir discriminant. Si la sensibilité de la copeptine, de la MR pro-ADM, de la pro-ET1 ou de la MR pro-ANP sont bonnes (voire excellentes pour les deux premières), c'est au prix d'une spécificité médiocre. Devant ces résultats, il parait difficile d'essayer de trouver une combinaison de certains biomarqueurs pour améliorer ces données. En effet, combiner deux marqueurs très sensibles et peu spécifiques aura pour effet de réduire drastiquement le taux de faux négatif, mais aussi de vrais positifs. Ainsi, les VPN obtenues ne pourraient être intéressantes du fait d'un trop large intervalle de confiance pour le premier, et assorties d'une VPP médiocre.

En outre, pour ces cinq biomarqueurs, il existe 10 combinaisons possibles de deux marqueurs, 10 de trois, cinq de quatre, soit au total, 25 combinaisons testables. Contrairement à l'étude d'Eurlings et al. [148], où chaque marqueur était indépendamment associé au critère de jugement, avec des AUCROC correcte, dans le cas de l'étude BIODINER il parait ainsi inutile de tester différentes combinaisons. Les analyses préliminaires que nous avons conduites vont dans ce sens.

On notera l'analogie de ce cas de figure avec celui de notre étude BISTRO, qui sous tend un principe qui semble logique et intuitif : l'association de biomarqueurs non discriminant peut difficilement donner de bons résultats. Nous ne connaissons pas d'exemple dans la littérature où le dosage combiné de plusieurs biomarqueurs a des caractéristiques intéressantes, alors que chacun individuellement n'en avait pas.

D. Perspectives générales

En 2006, RS Vasan recensait plus d'une trentaine de biomarqueurs dosables d'intérêt potentiel dans l'évaluation du risque cardiovasculaire [140]. Près de dix ans plus tard, et sur l'ensemble des pathologies rencontrées aux urgences, c'est plus d'une centaine de biomarqueurs qui pourraient être utiles en pratique clinique. Le nombre potentiel de combinaisons à évaluer est ainsi astronomique. Comme nous avons pu l'observer avec l'étude BISTRO, il apparaît indispensable que les marqueurs aient fait la preuve de leur intérêt en dosage singulier avant d'envisager de les évaluer de manière conjointe avec d'autres. Dans le cas précis de l'évaluation des crises convulsives, l'approche multimarqueur parait vaine, en l'absence de candidat singulier potentiel. En revanche, l'étude BISTRO nous a permis de relever des variables cliniques d'intérêt pouvant améliorer la prédiction du risque d'aggravation. Ceci nous rappelle bien sûr la valeur primordiale et prépondérante de l'évaluation clinique avant tout réflexion biologique.

L'avenir réside d'ailleurs probablement aussi dans l'association de critères cliniques et biologiques, intégrant ainsi dans les performances diagnostiques de nos modèles les variables cliniques, évidemment indispensables. Ainsi, Eurlings et al. ont présenté des résultats très intéressants en combinant quatre biomarqueurs dans le pronostic des dyspnées aigus aux urgences. Mais ils ont présenté dans leur même étude un modèle qui allie cette combinaison de biomarqueur avec des variables cliniques. Ainsi, le score Multimaker Emergency Dyspnea Risk Score (MARKED-risk) attribue un point pour chaque biomarqueur positif, et un point pour les critères cliniques suivant : âge > 75 ans, antécédent d'insuffisance cardiaque, dyspnée de repos,

pression artérielle systolique < 110 mmHg. La discrimination de ce score est meilleure que celle basée uniquement sur les biomarqueurs (c-statistique 0.85, IC 95% 0.81 – 0.89), et sa prédiction rejoint presque la mortalité réellement observée.

De même, Jimenez et al. ont associé l'approche multimarqueur (Troponine et NT pro-BNP) avec le score clinique PESI (pulmonary embolisme severity index) et l'échographie doppler veineux des membres inférieurs dans la suspicion d'embolie pulmonaire. L'association de ces différentes variables permet une bonne stratification du risque, avec en outre une VPN supérieure à 99% pour une évolution compliquée dans l'embolie pulmonaire [149].

II) Combinaison linéaire de deux biomarqueurs

Nous allons évaluer ici l'approche de Su et Liu pour trouver la BLC, la combinaison linéaire qui maximalise l'aire sous la courbe ROC [65].

On rappelle que sous des conditions de distribution gaussienne, la combinaison de deux biomarqueurs A et B A+αB aura l'aire sous la courbe ROC maximale pour

$$\alpha = b/a = \frac{\sigma^2{}_A.\hat{\mu}_B - \sigma_{AB}.\hat{\mu}_A}{\sigma^2{}_B.\hat{\mu}_A - \sigma_{AB}.\hat{\mu}_B}$$

A. PCT et Lactate

On cherche ici à évaluer l'AUC ROC du marqueur X=PCT+αLactate. On détaille d'abord le calcul pour la prédiction du critère de Sepsis sévère. En conservant l'hypothèse de matrices de covariances proportionnelles entre malades et contrôles, on obtient α=1,7. On s'intéresse donc à présent au marqueur

$$C = PCT + 1{,}7 * Lactate$$

La courbe ROC obtenue est reproduite ci-dessous, avec celles respectives de la PCT et du lactate :

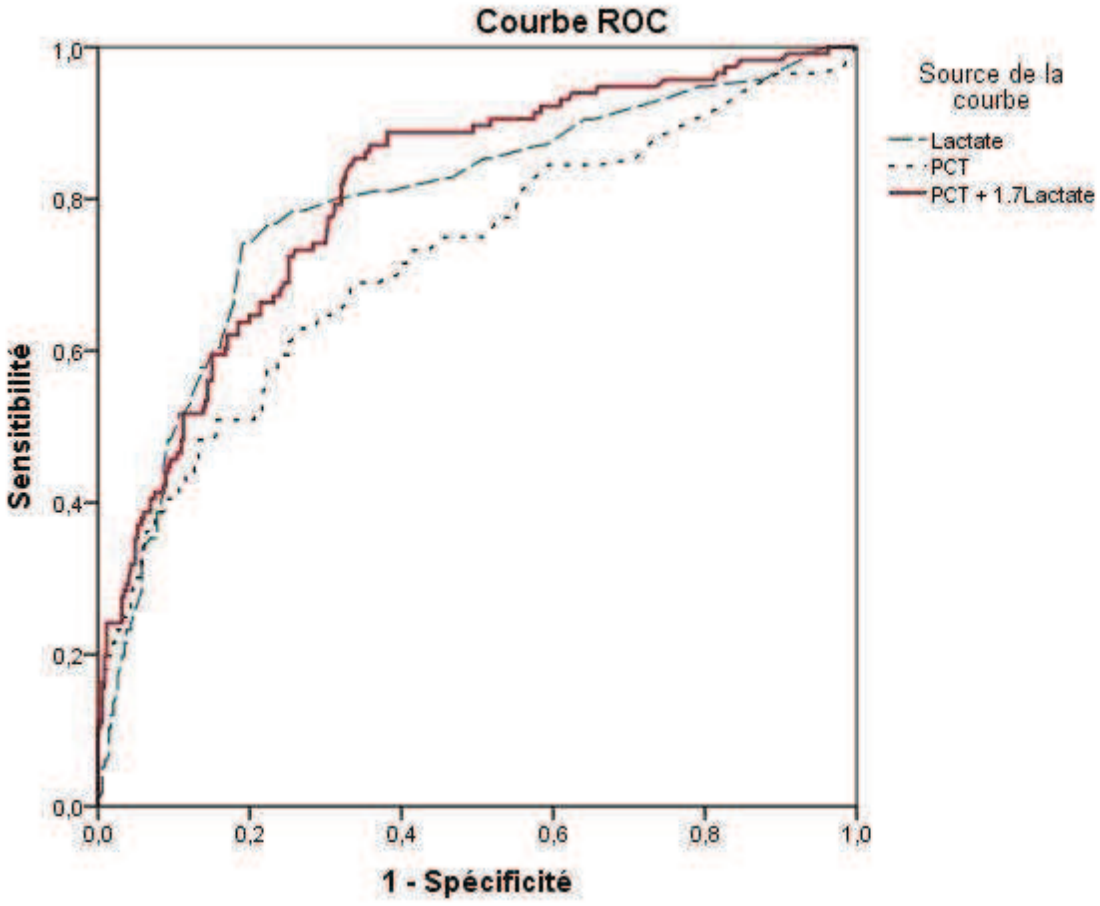

Figure 10 : Courbes ROC (Receiving Operator Characteristics) pour la prédiction du sepsis sévère – cohorte Freund et al. PCT/lactate. PCT : procalcitonine.

On retrouve ici les AUCROC exprimés précédemment de la PCT et du lactate pour la prédiction du sepsis sévère : 0,72 [0,66 – 0,78] et 0,79 [0,74 – 0,84] respectivement. L'AUCROC de la combinaison linéaire est ici supérieur à ces deux valeurs :

0,81 [0,76 – 0,86] (p=0.3 par rapport à la courbe ROC du lactate). Le gain ici retrouvé n'est pas significatif, mais confirme l'intérêt de rechercher une combinaison optimale. Ici, le seuil retrouvé par la méthode de Youden est à 3,43 – ce qui donne une sensibilité de 85% et une spécificité de 66%.

Le même calcul pour la prédiction du choc septique dans notre échantillon retrouve un coefficient α=0,1. Pour la variable C = PCT +0,1*lactate, on retrouve de même un gain d'AUCROC par rapport aux deux variables analysées séparément :

AUCROC = 0,90 [0,85 – 0,95] pour la combinaison linéaire, contre 0,84 [0,72 – 0,91] et 0,87 [0,74 – 0,93] pour le lactate et la PCT respectivement. Le gain d'aire sous la courbe n'est pas statistiquement significatif (p=0,20 vs PCT seul ou lactate seul).

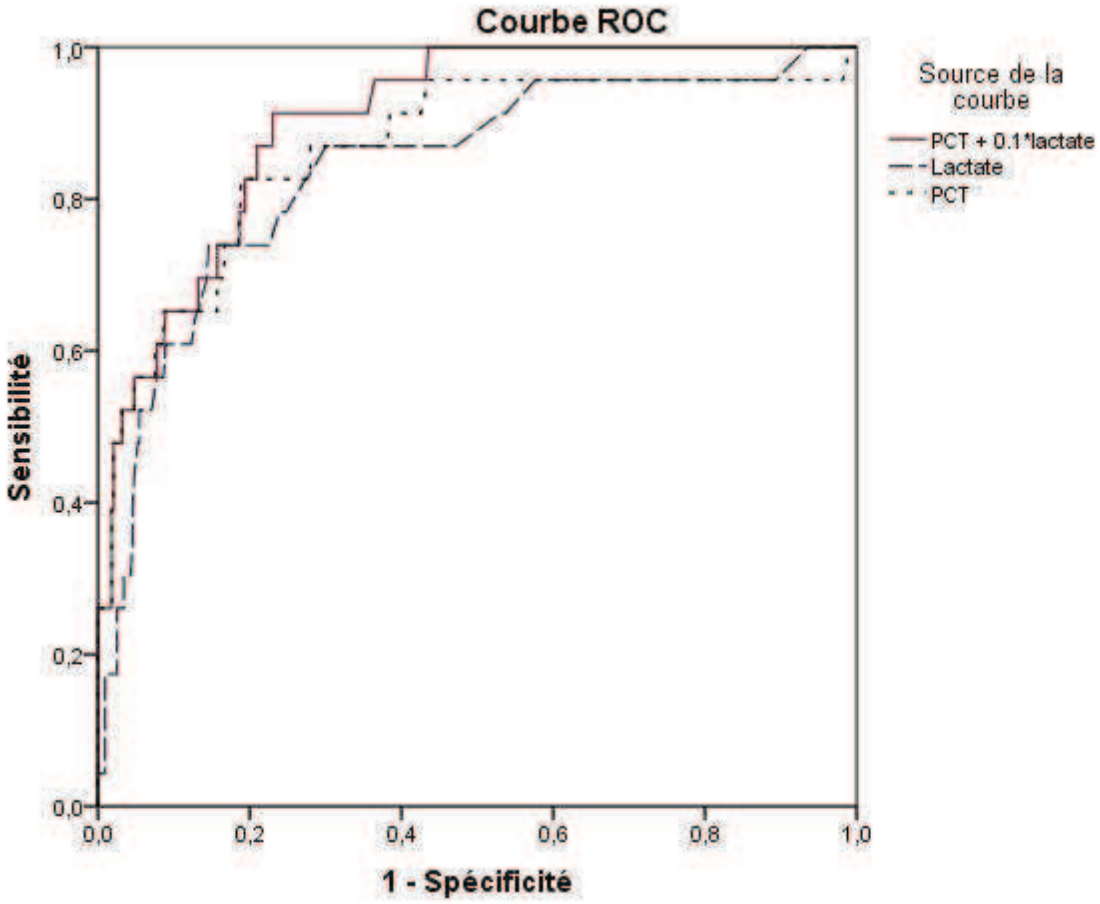

Figure 11 : Courbes ROC (Receiving Operator Characteristics) pour la prédiction du choc septique – cohorte Freund et al. PCT/lactate. PCT : procalcitonine.

Enfin, pour la prédiction de l'aggravation, (définie comme décès hospitalier ou admission en réanimation), la combinaison PCT+2.5*lactate a de même une meilleure discrimination que les deux marqueurs pris seuls, avec une AUCROC=0,71 [0,65 – 0,77] contre 0,66 [0,59 – 0,72] et 0,68 [0,60 – 0,73] pour la PCT et le lactate respectivement, (p=0,30 vs combinaison).

La méthode alternative qui consiste à attribuer comme coefficient a pour la variable A et b pour la variable B les valeurs des Odds ratios obtenus par régression logistique donne de moins bons résultats. Pour la prédiction du sepsis sévère, elle ne modifie que peu la courbe ROC (AUCROC = 0,81), mais pour le choc septique, elle fait moins bien que les variables séparées (AUCROC = 0,73). Ces résultats confirment l'intérêt de la méthode de Su et Liu par rapport à une approche plus classique, basée sur le poids des variables dans le modèle final de régression logistique.

B. Troponine et copeptine

En reprenant notre cohorte de patients des urgences avec une douleur thoracique [150] ainsi que les dosages supplémentaires de troponine hypersensible HsTnT (non publié, Appendice 1) , nous allons étudier les différentes combinaisons linéaires de la troponine conventionnelle (cTnI), de l'HsTnT et de la copeptine pour le diagnostic d'infarctus du myocarde (IDM), ou de syndrome coronaire aigu (SCA, incluant l'angor instable).

Pour le diagnostic d'IDM, les performances de la cTnI et de l'HsTnT sont déjà très élevées. Il y a ainsi peu à attendre d'une combinaison en termes d'AUCROC. Aussi, on s'intéresse à la prédiction du diagnostic de SCA, pour lequel les biomarqueurs étudiés sont moins performants. En appliquant la formule de Su et Liu, la BLC sera obtenue avec les coefficients suivant :

Copeptine + ***165*****cTnI* et *Copeptine* + ***0.54*****HsTnT* pour le diagnostic d'IDM

Copeptine + ***171*****cTnI* et *Copeptine* + ***0.68*****HsTnT* pour le diagnostic de SCA

On reproduit ci-dessous les différentes AUCROC obtenues pour ces différents diagnostics :

Diagnostic d'IDM	AUCROC	Intervalle de confiance à 95%	
Copeptine	0.73	0.66	0.82
cTnI	0.94	0.89	0.98
Copeptine + 165*cTnI	0.93	0.90	0.96
HsTnT	0.92	0.87	0.97
Copeptine + 0.54*HsTnT	0.92	0.88	0.95
Diagnostic de SCA			
Copeptine	0.69	0.62	0.77
cTnI	0.82	0.75	0.90
Copeptine + 171*cTnI	0.84	0.77	0.90
HsTnT	0.82	0.75	0.90
Copeptine + 0.68*HsTnT	0.83	0.76	0.90

Tableau 2 : AUCROC : Aire sous la courbe Receiving Operator Characteristics. cTnI : Troponine conventionnelle, HsTnT : Troponine Hypersensible.

La combinaison linéaire de la troponine et de la copeptine ne permet donc pas d'améliorer sensiblement l'aire sous la courbe ROC.

En utilisant les analyses complémentaires faites, dont le dosage de la heart fatty acid-binding protein (hFABP, Appendice 3), nous avons testé plusieurs combinaisons de biomarqueurs. En particulier, la formule de Su et Liu suggère que la BLC serait :

4,14*cTnI + **0,024***Copeptine + **0,87***hFABP

Cette combinaison permet le diagnostic du SCA avec une bonne discrimination, similaire à celle de la cTnI seule : on retrouve une AUCROC à 0,84 [IC 95% 0,77 – 0,90]

C. Protéine S100B et copeptine

Enfin, sur les mêmes bases de calcul, nous avons évalué la combinaison linéaire optimale de la S100B et de la copeptine dans le contexte des convulsions aux urgences pour prédire le risque d'aggravation. Ces deux marqueurs ont montré de faibles performances séparément, avec une AUCROC de 0,57 [IC 95% 0,51 – 0,64] pour la S100B et 0.59 [IC 95% 0,54-0,64] pour la copeptine. Ainsi, la combinaison Copeptine + 493*S100B a été évaluée mais ne donne pas de résultats meilleurs, avec une AUCROC de 0,59 [IC 95% 0,53 – 0,65].

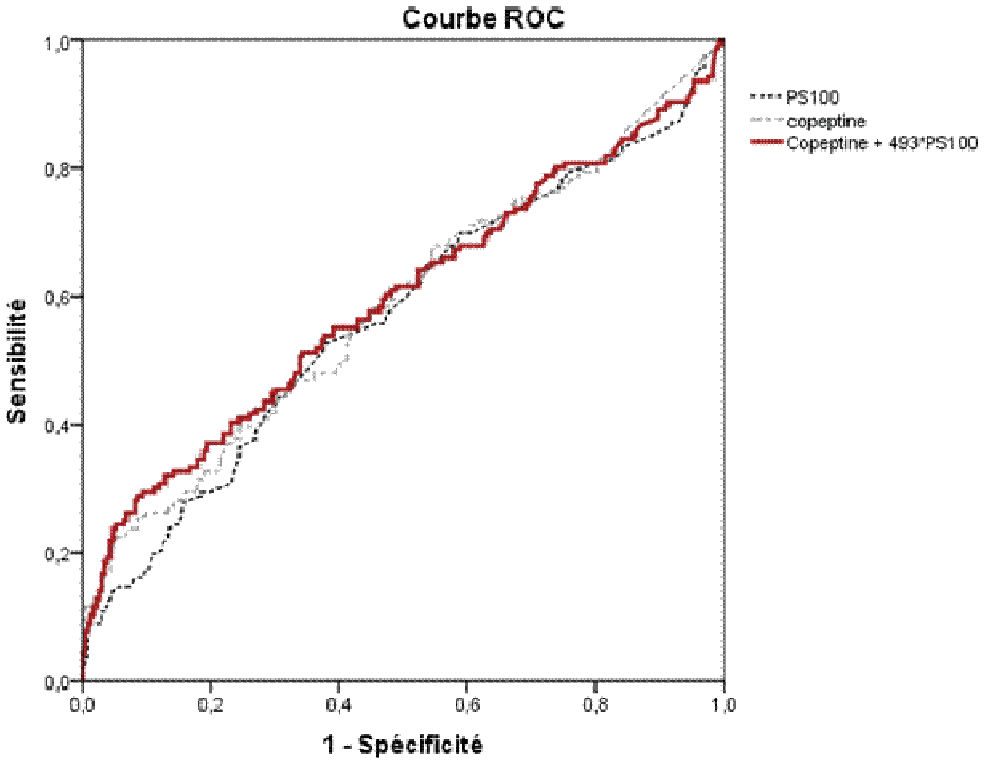

Figure 12 : Courbe ROC (Receiving Operator Characteristics) pour le diagnostic d'aggravation secondaire. Cohorte freund et al. de l'étude BISTRO

III) Limites de cette approche

Nos différents travaux illustrent les limites de l'approche multimarqueurs. Nous avons discuté plus haut des limites précises de chacune de ces études. Nous allons illustrer quelle peut être la valeur ajoutée d'une combinaison de biomarqueurs en fonction de leurs caractéristiques propres de sensibilité et spécificité. Considérons un biomarqueur très sensible : il sera positif pour la grande majorité des patients malades, et pourra donner de faux positifs chez les patients sains. On peut le représenter de la façon suivante :

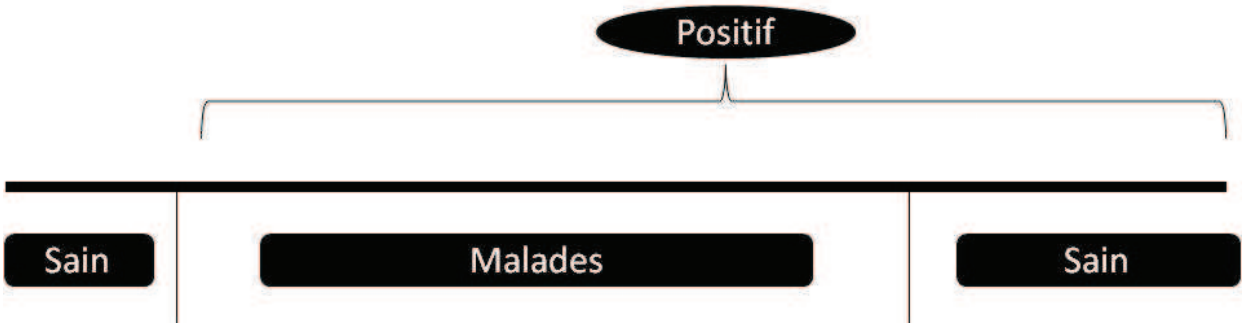

Figure 13 : illustration d'un biomarqueur très sensible. Tous les malades ont un résultat positif.

De même, un biomarqueur avec une grande spécificité pourra être représenté comme suit :

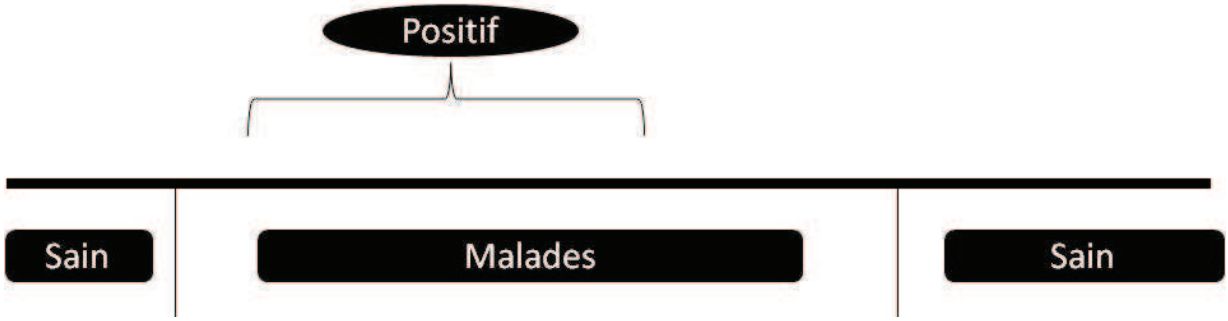

Figure 14 : illustration d'un biomarqueur spécifique. Tous les patients avec un résultat positif sont malades.

Ainsi, la combinaison de deux marqueurs, l'un fortement spécifique et l'autre fortement sensible n'aura vraisemblablement pas d'intérêt. En effet, les vrais positifs du marqueur spécifique auront été identifié par le marqueur très sensible (zone 1 figure ci-dessous), et ses dosages négatifs ne pourront être dissociés des vrais ou faux positifs du second marqueur (zone 2 et 3 respectivement) :

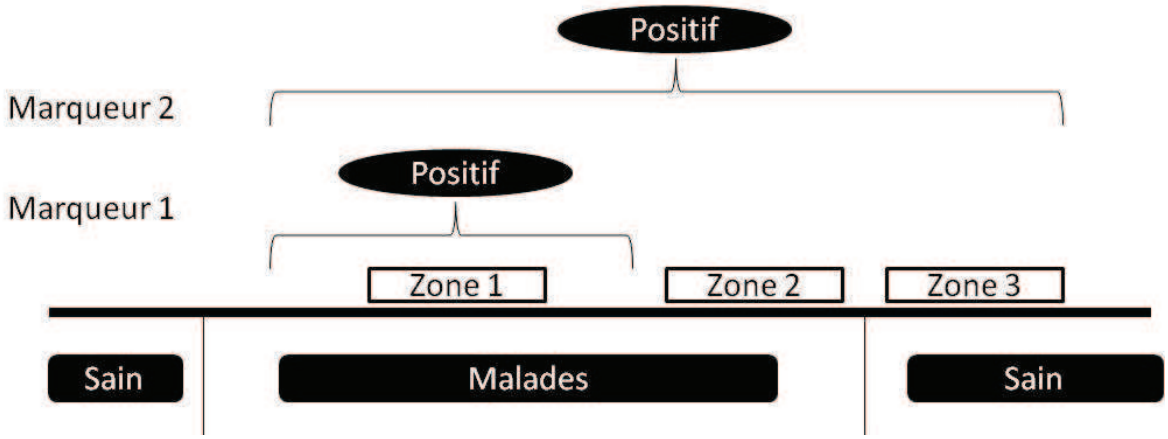

Figure 15 : Marqueur 1 : forte spécificité ; Marqueur 2 : forte sensibilité
Zone 1 : Vrai positif pour les deux marqueurs
Zone 2 : Faux négatif pour marqueur 1, vrai positif pour marqueur 2
Zone 3 : Vrai négatif pour marqueur 1, faux positif pour marqueur 2

Pour espérer avoir une combinaison de biomarqueurs intéressantes, il faut choisir en premier des marqueurs qui apporteront des informations complémentaires. On représente ci dessous un exemple théorique de deux marqueurs dont la combinaison aura une sensibilité améliorée : les faux négatifs de l'un étant des vrais positifs de l'autre. Il s'agit d'une représentation de ce qui a été utilisé pour améliorer la sensibilité dans le diagnostic du SCA : la troponine étant imparfaitement sensible, on a évalué un autre marqueur, la copeptine, très sensible aussi, qui se positive chez

les patients faux négatifs de la troponine. Ainsi, la sensibilité que l'on obtient pour les associations Copeptine/troponine est excellente, mais au prix de sa spécificité comme on le voit ci-dessous, du à l'augmentation du nombre de faux positifs.

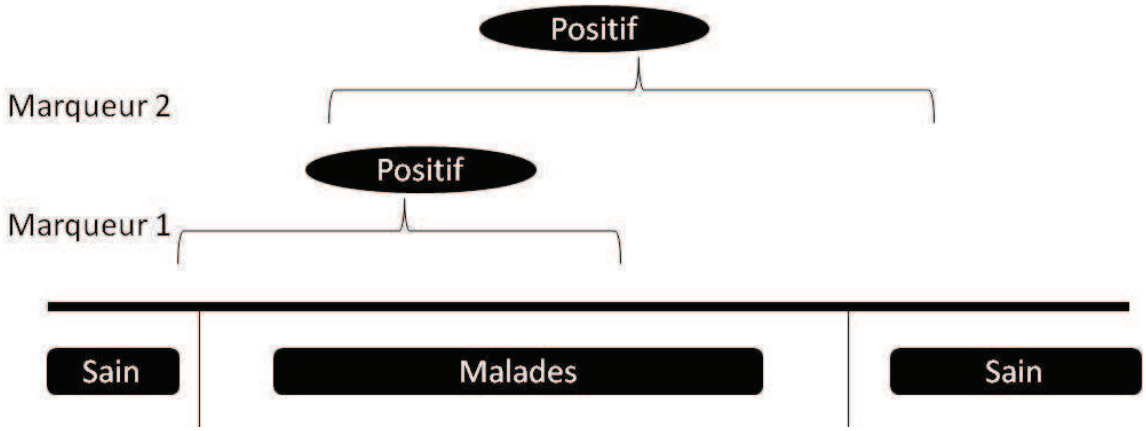

Figure 16 : illustration d'une association de deux marqueurs qui augmente le nombre de faux positifs.

Ainsi, on retire deux limites importantes à cette approche : d'une part, il est impératif que les marqueurs testés ne soient pas redondants, et d'autre part, l'amélioration d'une des caractéristiques fondamentales (sensibilité ou spécificité) se fera vraisemblablement au détriment de l'autre. Ainsi, même si la sensibilité de l'approche combinée de la troponine (cTnI ou HsTnT) et de la copeptine est significativement meilleure, il ne nous a pas été possible de démontrer une amélioration de la discrimination, estimée par la valeur de l'aire sous la courbe ROC.

D'autre part, trivialement, pour qu'une association de biomarqueurs soit performante, il est nécessaire que chacun d'entre eux le soit. Ainsi, lorsque nous avons cherché à évaluer le dosage de la copeptine et de la S100B, l'hypothèse sous-jacente pour évaluer la combinaison des deux était que ces marqueurs étaient dotés de

performances diagnostiques et pronostiques correctes – comme cela a été suggéré dans la littérature dans d'autre contexte que les convulsions. Malheureusement, avec une aire sous la courbe ROC inférieure à 0,6 pour ces deux marqueurs, il est peu probable qu'il existe une combinaison permettant d'obtenir de bonnes performances. Ainsi, que ce soit par combinaison linéaire ou score, aucune approche n'a permis d'obtenir des résultats satisfaisants. Il en a été de même pour l'étude BIODINER (appendice 2), dans laquelle aucun des marqueurs testés n'était indépendamment associé au critère de jugement principal, et dont les performances diagnostiques étaient médiocres. Aucune combinaison de ces biomarqueurs n'a paru donner de résultat satisfaisant.

Pour trouver une combinaison optimale, nous avons appliqué la formule de Su et Liu qui permet de trouver les coefficients de la BLC, combinaison linéaire maximalisant l'aire sous la courbe ROC. Cette formule a été dérivée d'un calcul avec comme condition d'application une distribution normale des valeurs du biomarqueurs dans une population saine et malade. En pratique, cette hypothèse n'est pas vérifiée dans nos études. Ceci est expliqué en partie par le fait que la limite de détection est proche du seuil pathologique dans les biomarqueurs que nous avons testés. Par exemple, la répartition des valeurs de troponine des patients sains est fortement biaisée car une grande majorité des patients sains présentent un résultat de troponine indétectable. Les résultats de la formule de Su et Liu en sont ainsi faussés. Perkins et al. ont proposé une méthode pour s'affranchir de cette limite qui implique de lourds calculs, que nous laissons au lecteur le soin de découvrir [151].

Enfin, il est important de se rappeler que les performances diagnostiques ou pronostiques découlent immédiatement de la matrice 2x2 de classification en vrais positifs/faux positifs/vrais négatifs/faux négatifs. Une bonne sensibilité est donc intrinsèquement liée d'une part au nombre de vrais positifs, mais aussi au dénominateur : le nombre de malade. Ainsi, sélectionner une population dans laquelle la prévalence de la maladie est plus élevée permettrait d'améliorer la sensibilité et la VPN (par exemple). Cette sélection peut se faire de manière concomitante par l'approche multimarqueur comme on a pu le voir. Mais il est aussi essentiel de valider des outils qui permettent de sélectionner une population précise, sur laquelle l'intérêt du biomarqueur est plus fort. Par exemple, le dosage de D-dimères dans l'embolie pulmonaire n'est recommandé qu'en cas de probabilité pré-test faible ou intermédiaire, pour limiter le risque de faux négatifs. A l'intérieur de cette population, la règle PERC (Pulmonary Embolism Rule Out Criteria) permet sur la base de 8 critères cliniques d'exclure le diagnostic d'embolie pulmonaire [152,153]. En appliquant cette règle avant le dosage de D-dimères, on exclut ainsi des patients sains, et on augmente donc le taux relatif de malade dans la population testée. Cette combinaison permet ainsi d'améliorer la stratégie diagnostique, en ciblant précisément la population qui bénéficie le plus du dosage de D-dimères.

Pour évaluer tout biomarqueur ou combinaison, il convient avant tout de sélectionner la population cible optimale, pour avoir des résultats fiables et applicables.

IV) Perspectives

Nous allons ici évoquer trois axes de perspectives et d'amélioration après ces travaux : l'évaluation de nouveaux biomarqueurs à combiner, l'association aux critères cliniques, et une approche statistique plus poussée.

Comme nous l'avons vu, une des principales limites de l'approche multimarqueur réside dans le choix des biomarqueurs. Dans le cas de l'étude BISTRO, les performances médiocres de la S100B et de la copeptine ont empêché la construction d'un modèle biologique utile à la stratification du risque. Il n'existe actuellement pas de biomarqueur reconnu pour l'évaluation du risque ou de la gravité d'une crise convulsive, et la S100B et la copeptine ne sont pas des bons marqueurs à utiliser en pratique clinique dans cette situation. Nous allons évaluer les performances diagnostiques d'autres biomarqueurs potentiellement intéressant dans cette pathologie : la neuron-specific enolase [146,154], l'ischemia modified albumin [145,155,156] ou la glial fibrillary acid protein [157,158]. Dans l'hypothèse ou un de ces marqueurs présenterait des caractéristiques intéressantes, il sera utile d'évaluer différentes combinaisons de ces marqueurs entre eux ou avec d'autres marqueurs. Ainsi, pour le diagnostic de SCA aux urgences, nous avons évalué l'apport de la myoglobine, de la troponine hypersensible et du heart fatty acid binding protein [16,159]. De nombreuses combinaisons peuvent être envisagées, mais les résultats obtenus avec l'association copeptine-HsTnT semblent suffisant pour le but recherché : une valeur prédictive négative proche de 100%.

Enfin, comme nous l'avons vu précédemment, de nouveaux biomarqueurs d'intérêt pour le sepsis comme la présepsine [135] ont été étudiés, et leurs combinaisons avec le lactate pourraient être utile dans l'aide à la prédiction du sepsis sévère ou de l'aggravation.

Pour améliorer la performance de nos modèles, il peut être judicieux d'associer les paramètres cliniques aux variables biologiques. En effet, à l'instar de l'étude de de Kruif et al. où les auteurs présentaient un modèle alliant PCT, CRP et présence de frisson, l'ajout de critères cliniques peut améliorer la prédiction du modèle [68]. Les modèles précédemment évoqués pourraient voir leurs performances améliorer en ajoutant des composantes cliniques. La méthode la plus simple pour ce faire consiste à attribuer un point dans un score pour chaque variable ressortant comme facteur indépendant d'une analyse multivariée, ou encore le poids de son odds ratio ajusté dans une combinaison linéaire.

De manière intéressante, nous avons appliqué cette stratégie pour la cohorte de l'étude BISTRO. Aucun biomarqueur n'était inclus dans le score, qui comprenait les quatre critères prédictifs indépendants de mauvais pronostic. Le modèle obtenu est reproduit ci dessous :

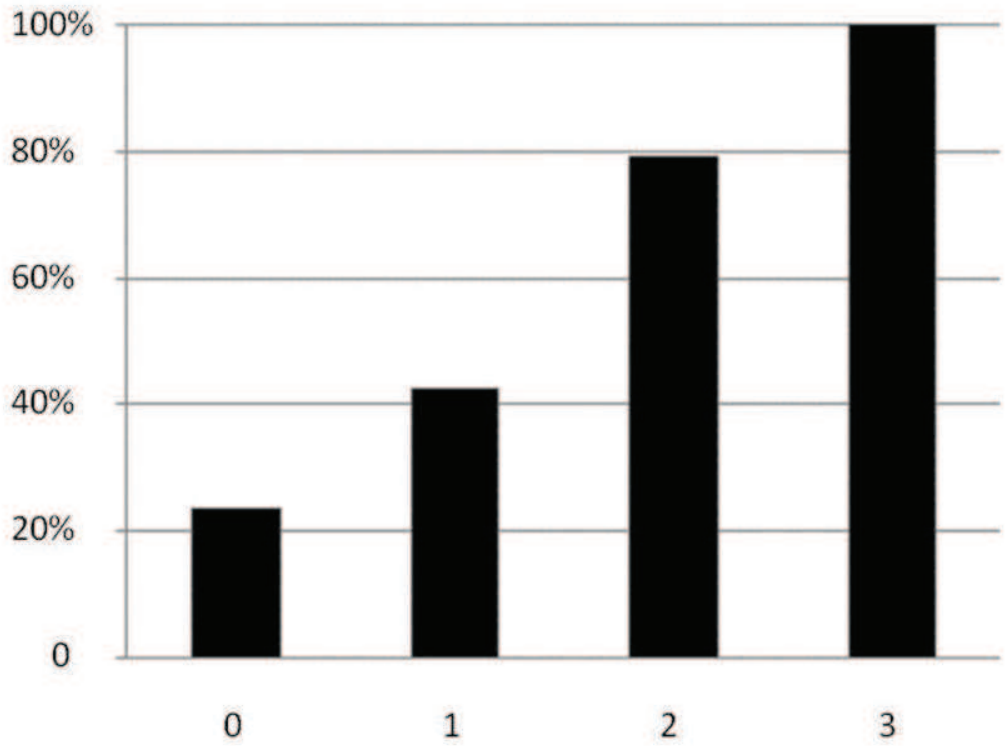

Figure 17 : Cohorte Freund et al de l'étude BISTRO :

Ordonnée : risque de récidive ou d'aggravation après une crise convulsive en fonction du nombre de paramètres cliniques présents (abscisse) parmi : age>40ans, crise provoquée, première crise, crise partielle complexe

Ce score qui a d'excellentes performances dans notre cohorte devrait être validé sur une cohorte externe afin de confirmer son intérêt et son applicabilité en pratique clinique.

Enfin, des outils statistiques puissant ont été développés et permettent de s'affranchir des conditions nécessaires à l'application de la formule classique de Su et Liu pour trouver la BLC, et pourraient être appliquées à nos cohortes. Pepe et Thompson ont décrit une méthode permettant de s'affranchir des conditions de normalité [67] et dont il serait intéressant d'appliquer les résultats à nos cohortes. Liu et Zhou proposent une formule pour deux biomarqueurs qui prend en compte la covariance pour optimiser

l'aire sous la courbe ajustée lorsque cette covariance est trop importante (comme c'est le cas pour l'HsTnT par exemple, ou la copeptine dans nos cohortes) [160].

Plutôt que de vouloir optimiser la courbe ROC dans son ensemble en maximalisant son AUC, il peut être préférable de ne s'intéresser qu'à une partie de cette courbe, lorsque par exemple seule une zone autour d'un seuil nous intéresse. Ainsi, la détermination d'une AUCpartielle (pAUC) et il est possible de trouver une combinaison qui optimise une partie seulement de la courbe ROC [161,162]

D'autres méthodes ont été décrites pour tenter de trouver la meilleure combinaison possible, et nous laissons au lecteur statisticien le soin de prendre connaissance par exemple de la méthode qui associe les valeurs minimales et maximales des biomarqueurs et s'affranchit aussi de leurs conditions de distribution [163], ou encore d'une méthode basée sur les indices de Youden des variables, décrite récemment par Yin et Tian [164,165].

CINQUIEME PARTIE :

Conclusions

Au travers de trois pathologies (le sepsis, le SCA, et la crise convulsive), nous avons étudié l'intérêt de l'association de différents biomarqueurs, et les différentes façons de les combiner.

Ces exemples nous ont montré plusieurs cas de figures qu'il convient de distinguer. Par exemple, pour le diagnostic de SCA, l'urgentiste recherchera une stratégie la plus sensible possible pour s'approcher le plus possible d'une VPN parfaite, et ainsi ne laisser sortir de l'hôpital aucun patient avec un infarctus. En conséquence, l'association de deux marqueurs très sensibles (copeptine et troponine), en prenant comme critère la positivité de l'un ou de l'autre permet d'obtenir de meilleures performances diagnostiques pour l'exclusion du SCA. L'excellente sensibilité de la HsTnT ne laisse pas de champ à la création d'un nouveau biomarqueur combiné qui la supplanterait. La copeptine permet ici de rattraper les rares faux négatifs de la HsTnT. Ainsi, la combinaison de ces deux marqueurs très sensible augmente encore un peu la sensibilité, mais au détriment de la spécificité.

A l'inverse, comme on l'a vu avec l'étude BISTRO, PS100 et copeptine sont tous deux de mauvais marqueurs dans l'évaluation de la gravité après une crise convulsive. L'association de deux mauvais marqueurs ne saurait en donner un correct, et les différentes méthodes d'associations testées n'ont pas permis d'améliorer notre stratégie et de parvenir à une combinaison correcte. Il en est de même pour l'étude BIODINER : les différentes associations de 5 biomarqueurs avec de mauvaises performances n'ont pu donner de résultat satisfaisant.

En revanche, nous avons vu que pour la prédiction du sepsis sévère ou du choc septique, la PCT et le lactate était chacun dotées de bonnes performances diagnostiques (sans être excellentes). Nous avons montré ici que la combinaison de la PCT et du lactate apportait plus d'aide au diagnostic que chacun d'eux séparément. Ainsi, nous avons pu construire une combinaison linéaire de ces deux marqueurs qui présente de meilleures performances diagnostiques dans la stratification des états septiques graves, avec en particulier une meilleure discrimination.

C'est probablement dans ce cas de figure que l'approche multimarqueur a le plus d'intérêt, et qu'il convient de poursuivre ce travail.

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Appendice 1 : HsTnt et Copeptine

Concomitant measurement of Copeptin and High sensitivity Troponin for fast and reliable rule out of acute myocardial Infarction : an ancillary study *(unpublished)*

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Abstract

Background:

Newer assays (high sensitive troponin T, HsTnT) and biomarkers (copeptin) recently improved the management of chest pain in the Emergency Department.

Objectives: To assess the negative predictive value (NPV) of the combination of HsTnT and copeptin for the diagnosis of acute myocardial infarction (AMI).

Methods: In consecutive patients presenting into three emergency departments with chest pain (<6 h) suggestive of AMI, HsTnT and copeptin were measured at presentation, blinded to the emergency physicians. The medical management of patients was left to the discretion of the attending physicians according to the suspected diagnosis, and result of conventional troponin I (cTn I). The discharge diagnosis was adjudicated by 2 independent experts using all available data.

Results: 317 patients were included. AMI was confirmed in 45 patients (14%), 13 of them were STEMI, and 32 NSTEMI. A copeptin level < 10.7 pmol/l in combination with a HsTnT < 0.014 µg/l correctly ruled out AMI with a higher sensitivity than cTnI : 1.00 (95% confidence interval: [0.90-1.00]) vs. 0.71 [0.55-0.84], p<0.001. We observed as well a significant gain in NPV: 1.00 [0.96-1.00] for copeptin + HsTnT vs. 0.95 [0.92-0.97] for cTnI alone (p=0.03).

Conclusion: Copeptin in association with HsTnT is a fast and reliable tool to rule out AMI, with a sensitivity and a NPV of 1.00 in our sample. Interventional studies are warranted to confirm these findings.

INTRODUCTION

Early identification of acute myocardial infarction (AMI) in the emergency department (ED) remains crucial, with approximately 15 million patients per year presenting to an ED in the United States for chest pain [1, 2]. Acute coronary syndrome (ACS) is the final diagnosis of chest pain in less than 20% patients [3], and electrocardiogram (ECG) is helpful in less than 30% cases [3, 4]. The sensitivity and negative predictive values (NPV) of troponin and ECG remain imperfect: conventional cTn failed to endorse this role-model position in the ED, as the delay for its elevation is of 4-6 hours [5-11]. The need for repeated cTn measurement is time and money consuming, and increases the work load of overcrowded ED [5-11]. Newer assays have been developed, and high sensitivity troponin (HsTn) has been associated with higher sensitivity and NPV than conventional cTn.

Copeptin, which is the c-terminal part of the vasopressine hormone,is a new biomarker of endogenous stress. Its combination with conventional cTn seems useful for a rapid rule out of AMI [12, 13]. Therefore, the objective of the present study was to evaluate the association of HsTn and copeptin for a rapid and reliable diagnosis of AMI.

PATIENTS AND METHODS

Setting

During the period from August 2005 to January 2007 in three urban teaching hospitals, we prospectively enrolled patients (> 18 years) presenting to ED with chest pain suggestive of AMI with onset or peak within the last 6 h. Patients with acute or chronic kidney failure requiring dialysis were excluded. The study was performed according to the principles of the Declaration of Helsinki and approved by the local ethics committee (Comité de Protection des Personnes Ile-de-France VI, CHU Pitié-Salpétrière Hospital, Paris, France). Because routine medical care was unchanged, waived informed consent was authorized. We followed the recommendations concerning the reporting of diagnostic studies, the Standards for Reporting of Diagnostic Accuracy (STARD) initiative [14]. This is a post hoc analysis of a previous published study [15].

Routine assessment

As part of the routine assessment in our institutions, all patients underwent an initial clinical evaluation that included clinical history, physical examination, 12-lead ECG, pulse oximetry, routine blood tests, and chest X-ray. Conventional cTnI was measured at presentation and, if needed, repeated after 3 to 9 h, as long as clinically indicated. Thus, according to the diagnosis of NSTEMI (non - ST elevation MI) or STEMI (ST elevation MI), the patients were admitted either to the cardiology unit for further evaluation and treatment or directly to the catheterization laboratory for primary percutaneous coronary intervention Emergency physicians in charge, and experts (see below) were blinded to the

results of HsTnT and copeptin, and biologists were blinded to the emergency diagnosis suspected by physicians.

To determine the etiologic diagnosis of chest pain at presentation for each patient, two independent experts (emergency physicians), reviewed all available medical records pertaining to the patient from the time of ED presentation to 30-day follow-up. When diagnostic disagreement occurred, cases were reviewed and adjudicated in conjunction with a third expert (also emergency physician).

AMI was diagnosed according to the joint European Society of Cardiology / American College of Cardiolology / American Heart Association / World Heart Federation Task Force re - definition -of MI guidelines [16]. Diagnosis of AMI required a cTnI increase (or a rise/fall pattern) above the 99th percentile, associated with at least one of the following: symptoms of ischemia, new ST-T changes or new Q wave on ECG, imaging of new loss of viable myocardium, or normal cTnI on admission. As the conventional cTnI methods (used routinely in our institutions) do not allow the measurement of 99th percentile with the precision required (see below), AMI was diagnosed on the basis of a cTnI value above the 10%CV level.

As not all patients had a second dosage of cTn, the change criteria (rise or fall) could not apply for every patients, leading that some of them had an adjudicated diagnosis based on experts reviewing with all required data (such as ECG, cardiac angiography, ect.)

Unstable angina was diagnosed in patients with constant normal cTnI levels and history or clinical symptoms consistent with ACS. Pre-defined further diagnostic categories included AMI (STEMI with the presence of ST-segment elevation in ≥2 continuous leads on electrocardiography or new onset of left bundle branch block, or NSTEMI), unstable angina, and a third group including cardiac but not coronary symptoms (e.g., stable angina, myocarditis, arrhythmias, heart failure), non - cardiac symptoms (e.g., pulmonary embolism), and chest pain of unknown origin.

Biochemical analysis

In two EDs (Cochin Hospital and La Pitié Salpêtrière Hospital), plasmatic cTnI concentrations were routinely measured on an X-pand® HM analyser, using the Cardiac Troponin I one-step enzyme immunoassay (Siemens Healthcare Diagnostics Inc., Newark, NJ). The measuring range extended from 0.04 to 40.00 µg/L. The threshold for this method (0.14 µg/L) corresponds to the lowest substrate concentration that can be reproducibly measured with a CV of ≤ 10%. In the remaining ED (Bicêtre Hospital), plasmatic cTnI concentrations were routinely measured on an Access® analyser (Beckman Coulter, Inc., Brea, CA). The measuring range of this one-step chemiluminescent immunoassay extended from 0.01 to 100.00 µg/L. The threshold value (10%CV) given by the manufacturer is 0.06 µg/L.

Copeptin was measured on a BRAHMS Kryptor system (Thermo Fisher Scientific). The assay has a reported analytical detection limit of 4.8 pmol/L and a functional assay sensitivity (lowest value with an

interassay CV 20 %) < 12 pmol/L that allows precise measurement of copeptin in a range of 4.8 to 1,200 pmol/L. Copeptin determinations were performed blinded to the clinical assessment of the emergency physicians, and to the experts.

Heparinized samples collected on admission and, if available, in sample collected 3 to 9 hours late, were analysed. Plasmatic HsTnT concentrations were measured on an Elecsys2010® analyzer using the HsTnT one-step electrochemiluminescence immunoassay (RocheDiagnostics, Meylan, France). The measuring range extended from 0.003 to 10 µg/L. The threshold value for this method is 0.014 µg/L and corresponds to the 99th percentile. CV was found to be < 10% at 0.014 µg/L.

In our laboratory, CVs obtained in Roche quality controls containing 0.027 and 2.360 µg/L of HScTnT were < 4 %. These analytical performances were in accordance to manufacturer's data. HScTnT measurement were performed blinded to the clinical assessment of the emergency physicians, and to the experts.

Statistical analysis

Continuous variables are presented as mean ± SD (for normally distributed variables) or median (25th – 75th percentile), categorical variables as numbers and percentages. Normality was assessed using Kolmogorov-Smirnov test. Continuous variables were compared with Student t test or Mann-Whitney *U* test and categorical variables using the Pearson chi-square test. Correlations among continuous variables were assessed with the use of the Spearman rank-correlation coefficient. The sensitivity, specificity, and positive (PPV) and negative predictive value (NPV) were calculated and their 95% confidence interval [95%CI] calculated. To compare the accuracy of the biomarkers to diagnose AMI, comparison of areas under the receiver-operator characteristic (ROC) curves (AUC_{ROC}) was performed [17]. Since different methods were used for cTn, the validity of the ROC curve might be discussed. Thus, we performed additional subgroup analyses according to the methods used.

As the ROC curve is now recognized as an insensitive method to evaluate the gain of biomarkers [17], the net reclassification index (NRI) method was used, as recently described [18]. For tests with binary outcomes (such as cTn for the diagnosis of AMI), NRI is defined as the gain in certainty of the first test (cTnI) minus the gain in certainty of the second test (HScTnT), or alternatively stated, the differences of the sum of the sensitivity and specificity:

$$NRI_{HScTnT\ vs\ cTnI} = (Sensitivity + Specificity)_{HScTnT} - (Sensitivity + Specificity)_{cTnI}$$

NRI is the combination of four components: the proportion of individuals with events who move up or down a category and the proportion of individuals with nonevents who move up or down a category. We provide a contingency table comparing diagnostic classification according to cTnI and hsTnT plus copeptin, with shifts between the two classifications in order to represent the possible benefit of this combination of biomarkers in terms of number of patients correctly reclassified.

All hypothesis testing were two-tailed, and a p value of < 0.05 was considered significant. Statistical analysis was performed using StatView for Windows (version 5.0) (SAS Institute, Cary, NC,) and MedCalc for ROC analysis (Medcalc software, Mariarkerke, Belgium). Graphs were built with GraphPad Prism 5 (Graphpad software Inc, La Jolla, CA).

RESULTS

Over 18 months, 317 consecutive patients were enrolled in the study. Baseline characteristics of patients are shown in Table 1. There was a significant proportion of patients with a prior history of cardiovascular events (26%, n=83). Chest pain was considered typical of ACS in 43% (n=136) of patients. AMI was confirmed in 45 patients (14%), 13 of them were STEMI, and 32 NSTEMI. At 30 days, there were 3 deaths (two in the AMI group and one in the other cause group), and 4 relapses of ACS, all in the AMI group.

Copeptin and HsTnT diagnostic performances compared to that of conventional cTnI

The 2 ROC curves (for each assay) had a similar AUC for the diagnosis of AMI : 0.93 [0.87-0.98] and 0.97 [0.94-1.00] (NS), compared to 0.92 [0.88-0.94] for the combination of HsTnT and copeptin (p=0.30) (Figure 1).

Table 2 shows the added value of combination of a positive HsTnT and/or a positive copeptin versus cTnI alone. A copeptin level < 10.7 pmol/l associated to a HsTnT level < 14 pmol/l correctly ruled out AMI with a sensitivity of 1.00 (95% CI: 0.90-1.00) vs. 0.71 (95% CI: 0.55-0.84) for cTnI (p<0.001), and a NPV of 1.00 (95% CI: 0.96-1.00) vs. 0.95 (95% CI: 0.92-0.97) for cTnI (p=0.023). However, the combination of HsTnT and copeptin did not significantly increase the sensitivity or NPV, compared to that of HsTnT alone.

NRIs and reclassification table are presented in Table 3. If the combination of copeptin with HsTnT increases sensitivity at admission (45/45 vs. 13/45), NRIs indicate that there was no significant gain in certainty using the combination in comparison to cTnI alone (Table 3).

Discussion

For decades, cTn has been the preferred marker for AMI diagnostic, a position re - affirmed in recent consensus guidelines [19, 20]. Recently, newer assays and biomarkers have been developed, but neither HscTnT, nor copeptin succeed in becoming the ideal biomarker, the one that would help physicians to set an early discharge policy for non cardiac chest pain, without any other investigation, especially as interventional studies are still lacking. Previous studies have clearly demonstrated that high and ultra sensitive cTn are more sensitive than conventional cTn [19-21]. Our previous published results also confirmed these findings [15]. However, the gain in NPV and sensitivity was slight and did not translate into a real clinical gain, as patient with negative values at their admission into the ED cannot be safely discharged, and repeated measurements as still necessary. Copeptin is a very sensitive, non-specific biomarker of endogenous stress, that has been demonstrated to be useful in

many situations, including assessment of chest pain [22-27]. Its rapid rise in the blood, may allow emergency physician to rule out of AMI in ED, when both copeptin and conventional cTn are negative.

Combination of these two biomarkers, i.e. HsTnT and copeptin, has only been evaluated in one recent study [28]: Giannitisis et al. found an incrementive value of copeptin to HsTnt for ruling out NSTEMI in patients presenting to a chest pain unit, with a NPV of 99% [97-100]. In our multicentric prospective study, we confirm the great benefit of concomitant measurement of Copeptin and HsTnT versus conventional cTnI. With a threshold of 10.7 pmol/l, defined by the ROC curve, sensitivity and NPV were of 1.00. This result suggests the possibility of an early and safe rule out strategy. Although the AUC of cTnI and the combination of HScTnT and copeptin were not significantly different, table 2 shows that the combination leads to a better recognition of patients with AMI. However, this encouraging finding needs to be confirmed by other studies, especially by interventional studies. With conventional management of chest pain investigation in the ED, patients cannot be discharged after a sole troponin measurement as the delay to its elevation is of 4-6 hours. Even with high sensitivity assays, the enhanced NPV failed to reach perfection, preventing physicians to rely only on this measurement.

When comparing this combination to conventional cTnI, we found a worsened AUC, although not significant. The diagnostic performances seem better with cTnI, however this is not of great interest for emergency physicians: the key to improve management of chest pain in the ED is to upgrade the sensitivity, and especially the NPV for AMI. Of note, we found in our study better performances of cTnI than in some previous cohorts that can be explained by several factors. First we used cTnI instead of cTnT, with a different assay than previously described, so our comparator could have better analytical properties. Although we found a higher AUC for cTnI, its value was included in the 95% CIs of the AUC of other cTn ([0.81-0.98] for the Christ et al. study [29] for example). We can also explain this discrepancy by our different inclusion criteria from those used by Keller et al. and Reichlin et al. [12, 19] who included patients with a chest pain of less than 12 hours, and with a higher rate of AMI and UA. Our population is therefore different from previous described ones, and that can cause different performances.

We previously described that HsTnT represents a slight gain for emergency physicians, with a better sensitivity and NPV only in patients with low to moderate pre-test probability [15]. This enhancement was too slight and prevented us to change clinical daily practice. This present study not only shows a perfect NPV (1.00), but also a significant incremental value to cTnI regardless the pre-test probability in terms of NPV and sensitivity. In overcrowding ED, where chest pain is a common admission cause, a very high NPV could be of great help.

Limitations

Our study presents some limitations. First, we only performed a single measurement of HsTnT and copeptin, and did not evaluate their kinetics, which would have been interesting, as previously suggested [30]. However, emergency physicians are looking for a very early and safe discharge as represented by a single measurement at admission. Second, we used two different assays for cTnI as the comparator, with a high diagnostic performance in our study, compared to that previously published. The ROC curve for the cTnI is then a combined ROC curve of two different assays making it questionnable. However, when constructing the ROC curves according to the two methods of measurement, we observed comparable results. Third, our study is observational and the 95%CI of sensitivity and NPV of the combination of HScTnT and copeptin are wide and can not allow to a definitive conclusion in patients with a high risk of AMI. Fourth, in our study the prevalence of AMI was low compared to that of previous studies, which could influence the result of the NPV [17].

Lastly, the recommended change criteria was not systematically used for all patients. Only 198 (63%) patients benefit from a second dosage of cTn. As a consequence, we may have overestimated the rate of AMI, and underestimated the rate of other diagnosis which could partially explain the low percentage of unstable angina (3%) compared to that of previous studies. Further studies with high risk patients or higher prevalence of AMI as final diagnosis are warranted to confirm this almost perfect NPV of the combination HScTnT and copeptin.

Conclusion

Negative HscTnT and copeptin would allow emergency physicians to rule out at admission the diagnosis of AMI. Interventional studies are warranted to demonstrate that use of the combination of HScTnT and copeptin may improve the management of chest pain patients in overcrowded ED.

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Table 1: Baseline characteristics of the population according to the pre - test probability (PTP).

	All patients	Low or moderate PTP	High PTP	p *
N	317	258	59	
Age (years)	57 ± 17	56 ± 17	60 ± 17	0.168
Men	205 (65)	166 (64)	39 (66)	0.88
Systolic BP (mmHg)	141 ± 28	141 ± 27	144 ± 30	0.396
Diastolic BP (mmHg)	80 ± 16	80 ± 16	82 ± 16	0.428
Heart rate	85 ± 45	84 ± 23	80 ± 19	0.177
Pulse oxymetry (%)	97 ± 3	97 ± 3	97 ± 2	0.051
TIMI Score	1 (0 – 3)	1 (0 - 2)	2 (1 – 4)	<0.001
Familial history of CAD	100 (32)	77 (30)	23 (39)	0.161
Personal history of CAD	83 (26)	56 (22)	27 (46)	0.0003
Dyslipidemia	113 (36)	86 (33)	27 (46)	0.069
Smoking	128 (40)	99 (38)	29 (49)	0.145
Diabetes	44 (14)	31 (12)	13 (22)	0.059
Hypertension	116 (37)	89 (34)	27 (46)	0.134
History of heart failure	21 (7)	14 (5)	7 (12)	0.083
Typical thoracic pain	136 (43)	105 (41)	31 (53)	0.11
Patients with positive cTnI at admission **	41 (13)	24 (9)	17 (29)	<0.001
Median eGFR (in ml/min/1.73m²)	77 (62 – 94)	77 (64 – 94)	76 (56 – 91)	0.107
Treatment received in the first 24 hours of admission:				
Aspirin	119 (38)	79 (31)	40 (68)	<0.001
Clopidogrel	54 (17)	29 (11)	25 (42)	<0.001
LMWH	68 (21)	41 (16)	27 (46)	<0.001
Anti-GPIIb/IIIa	3 (1)	1 (0)	2 (3)	0.09
Coronarography	83 (26)	51 (20)	32 (54)	<0.001
Outcomes				
Hospital-admission	192 (61)	140 (54)	52 (88)	<0.001
Admission in CCU	134 (42)	88 (34)	46 (78)	<0.001
Final diagnosis				
AMI	45 (14)	22 (9)	23 (39)	<0.001
STEMI	13 (4)	0 (0)	13 (22)	<0.001
NSTEMI	32 (10)	22 (9)	10 (17)	<0.001
Unstable angina	11 (3)	4 (2)	7 (12)	<0.001
Other diagnosis ***	261 (82)	232 (90)	29 (49)	<0.001

AMI, acute myocardial infarction; BP, blood pressure; CAD, coronary acute disease; eGFR, estimated glomerular filtration rate; LMWH, low molecular weight heparin;

CCU, Cardiologic care unit; STEMI, ST elevated myocardial infarction. Results are in mean ± SD, median (25^{th} – 75^{th} percentile), or number (percentage)

**: >0.14 µg/L in PS and CCH, > 0.06 µg/L in KB.

*** including: stable angina (n=63), pulmonary embolism (n=16), myopericarditis (n=43), heart failure (n=5) and others.

Table 2 : Diagnostic accuracy of HsTnT and copeptin

All Patients (n=317)	Sensitivity	Specificity	PPV	NPV	Acc
Positive cTnI	71 [55-84]	97 [94-98]	78 [62-89]	95 [92-97]	93 [90-96]
Positive HsTnT:	93 [80-98]	82 [77-87]	47 [36-58]	99 [96-100]	84 [79-88]
Positive HsTnT and/or copeptin :	100 [90-100]$	48 [42-54]*	24 [18-31]*	100 [96-100]$	56 [50-61]*

PPV: positive predictive value; NPV: negative predictive value; Acc: diagnostic accuracy; Values are expressed as a percentage and their 95%CI

Positive HScTnT: >14 ng/L; positive copeptin: >10.7 pmol/L

*, p<.001 versus positive HsTnT; $, p<.05 versus positive cTnI;

Table 3 : Contingency table

	AMI	no AMI	total	
Positive cTnI	32	9	41	
Negative cTnI	13	263	276	
	45	272		317
Positive HsTnT and/or Copeptin	45	141	186	
Negative HsTnT and copeptin	0	131	131	
	45	272		317

AMI : Acute Myocardial infarction

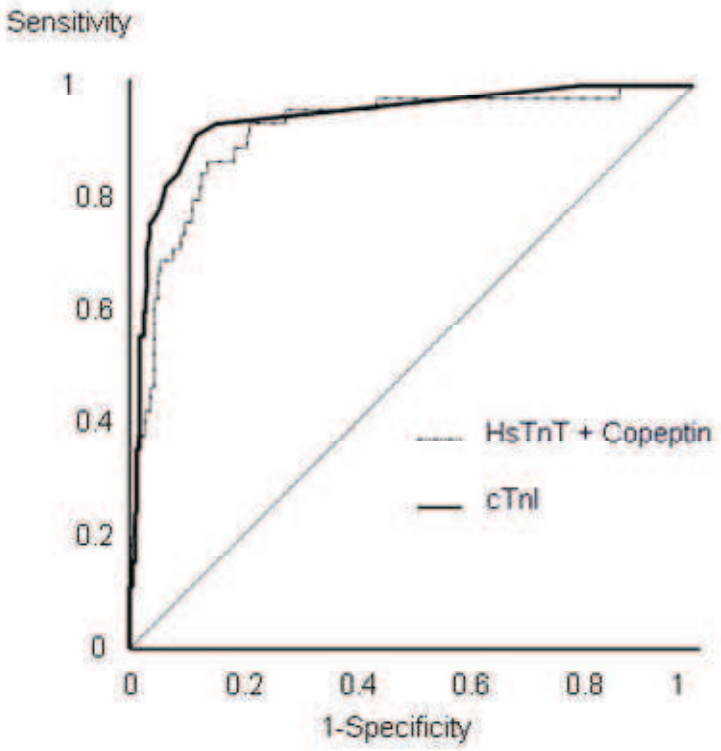

Fig 1: ROC curve for HsTnT+Copeptin and cTnI., for the diagnosis of AMI

Appendice 2 : Etude BIODINER

Prognostic value of pro-hormone-type biomarkers for severe acute dyspnea in the emergency department: the BIODINER study

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Author's contribution : PH, YEC, PR, BR designed the study. AM, LMJ, JYL, GDR, CL, PH, YEC, PR recruited the patients. BR and YF performed the statistical analysis and interpretation of data. PH wrote the manuscript. All authors revised the manuscript.

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Running title: pro-hormone biomarkers in severe acute dyspnea

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At a glance commentary: The risk stratification of acute dyspnea mostly relies on the presence of clinical severity criteria and on the results of blood gas, both lacking sensitivity and specificity. This study reports the prognostic usefulness of several pro-hormone-type biomarkers (procalcitonin, pro-adrenomedullin, copeptin, pro-endothelin 1, and pro A natriuretic peptid,) in a cohort of severe acute dyspnea patients in the emergency room. In our sample these biomarkers have a low added value to clinical signs for the prediction of a poor outcome.

Abstract

Rationale: Acute dyspnea is a frequent complaint in patients attending the emergency department (ED) with a wide range of causes and outcome profiles.

Objective: to evaluate the accuracy of pro-hormone type biomarkers (procalcitonin PCT, pro-adrenomedullin MR-proADM, pro-vasopressin copeptin, pro-endothelin 1 CT-proET-1, and pro A natriuretic peptid, MR-proANP) for the risk-stratification of severe acute dyspnea patients presenting to the ED.

Methods and measurement: This was a multicentre prospective observational study in 5 academic EDs. Adult patients with a chief complaint of acute severe dyspnea were recruited, and followed up for 30 days. Pro-hormone type biomarkers concentrations were measured on arrival. Combined primary endpoint was a poor outcome defined as a composite of intensive care unit admission, invasive ventilation and death. Multivariate logistic regression was performed to assess independent predictors of poor outcome.

Main results: 394 patients were included and analyzed, the mean age was 75± 15 year and 51% were male. One hundred and thirty seven patients (35%) met the combined primary endpoint. All pro-hormone-type biomarkers concentrations but MR-proANP were higher in the poor outcome group although they exhibited a poor discrimination and low diagnosis performances. The presence of either paradoxical abdominal breathing (odds ratio 2.48 [95%CI: 1.31-4.68]) or cyanosis (odds ratio 3.18 [1.46-6.89]) at admission was significantly associated with poor outcome. MR-proADM was the only biomarker associated with the combined primary endpoint (odds ratio 1.44 [95%CI: 1.13-1.82], p=0.003).

Conclusion: In patients with severe acute dyspnea in the ED, pro-hormone type biomarkers measurements have a low added value to clinical signs for the prediction of poor outcome.

Word count: 256

Introduction

Acute dyspnea is a frequent cause of emergency admission worldwide, accountering for up to 7 % of emergency department (ED) visits (1,2). The care of dyspneic patients in the ED presents two major challenges: identifying the etiology of the acute episode and risk-stratifying the severity of the patient to guide the decision to admit. . Moreover, there are numerous causes of acute dyspnea associated with a wide range of outcome profiles (2,3),. The most frequent causes of acute dyspnea are acute heart failure (AHF), community acquired pneumonia (CAP), acute exacerbation of chronic obstructive pulmonary disease (AECOPD) and pulmonary embolism (PE). Currently, the risk stratification of acute dyspnea mostly relies on the presence of clinical severity criteria and arterial blood gas analysis, both lacking sensitivity and specificity (4,5).

Recently, several pro-hormone-type biomarkers have been studied and exhibit promising results for the risk stratification in various clinical situations: sepsis, acute coronary syndrome, CAP, AECOPD, and AHF (3,5–19). These biomarkers comprise procalcitonin (PCT), midregional pro-A-Type natriuretic (MR-proANP), midregional pro-adrenomedullin (MR-proADM), pro-vasopressin (AVP, copeptin) and pro-endothelin1 (CT-proET1). However, their prognostic values have been mostly studied in specific diagnoses and not extensively in patients who share a similar complaint, which reflects the daily clinical practice in the ED.

In this study, we hypothesized that pro-hormone-type biomarkers may have an incremental added value to usual clinical variables to predict poor outcomes in patients presenting to the ED with severe acute dyspnea.

Study design, patients and methods

The BIOmarkers for Dyspnea IN Emergency Room (BIODINER) was a multicenter prospective observational study conducted in 7 academic emergency departments in France (Pitié-Salpêtrière, Cochin and Hôtel-Dieu hospitals (all in Paris), Rouen, Lille, Poitiers and Nantes). The primary objective was to evaluate the prognostic value of PCT, MR-proANP, MR-proADM, copeptin and CT-proET1 measured in blood at ED's admission in patients with severe acute dyspnea. Secondary objectives were to determine the best thresholds of the biomarkers studied for the prediction of the severity of the acute dyspnea episode and to study the accuracy of PCT and pro-ANP for the diagnosis of CAP and AHF respectively. Participants were informed about the study and written signed consent was waived because of the observational design (CCTIRS, Comité consultatif sur le traitement de l'information en matière de recherche dans le domaine de la santé, dossier 09.650bis, approved on January 14th 2010).

The statistical plan of the study was decided before the statistical analysis. The study was conducted according to Good Clinical Practice standards and the Helsinki Declaration, and the protocol was registered at ClinicalTrial.gov (NCT NCT01227317). We followed the STARD recommendations for reporting diagnostic studies (20).

Patients

Patients 18-year aged or older were eligible for inclusion if 1) they presented to one of the participant EDs with acute dyspnea as the chief complaint, associated with at least one of the following severity criteria: a respiratory rate higher than 25/min; a partial arterial oxygen pressure (PaO2) lower than 70 mmHg; a partial arterial carbon

dioxide pressure (PaCO2) higher than 45 mmHg and an arterial pH below 7.35; or a peripheral oxygen saturation (SpO2) less than 93% and 2) the main suspected etiology of dyspnea comprised one of the following: AHF, AECOPD, CAP, PE.

Patients were not included if they were under 18-years-old, or were unable to be contacted at day 30 (homeless) or if acute dyspnea was in relation with influenza, thoracic trauma, pneumothorax, anxious manifestation or asthma. Patients were secondarily excluded if no blood sample was drawn or no sample was available for pro-hormone-type biomarker measurement.

Intervention and blood sampling

After enrolment, clinical and physiological data were recorded on an electronic case report form (eCRF, Telemedicine Technologies, Boulogne, France) together with the usual biological variables (blood cell count, arterial blood gas, serum creatinin). The care of the patient was left to the discretion of the consulting physician for the diagnosis process and treatment. The patients were followed up at day-30 via the medical file and/or a phone call if they were discharged home before day 30.

During the first blood sampling in ED, a venous blood sample was withdrawn, collected in EDTA tubes and sent to each local biochemistry laboratory. For the pro-hormone-type biomarkers, plasma aliquots were immediately centrifuged and stored at -20℃ until further analysis. At the end of enro lment, all samples were measured in each laboratory for pro-hormone-type biomarkers. Biologists were blinded for clinical information and emergency physicians were blinded for pro-hormone biomarker results.

PCT, Copeptin, MR-proADM , MR-proANP and CT-proET-1 were measured in batches using the immunofluorescent assays (Kryptor, BRAHMS Biomarkers,

ThermoFisher Scientific, Hennigsdorf, Germany). Kryptor® is an automated immunofluorescent analyzer using the Time Resolved Amplified Cryptate Emission (TRACE) technology. For CT-proET-1 a kit for research was used. Analytical detection limits were respectively 0.02 µg/L for PCT, 4.8 pmol/L for copeptin, 0.05 nmol/L for MR-proADM, 2.1 pmol/L for MR-proANP and 2.94 pmol/L for CT-proET-1. The functional sensitivities (CV20%) were respectively: 0.06 µg/L for PCT, 12 pmol/L for copeptin, 0.25 nmol/L for MR-proADM, 10 pmol/L for MR-proANP and 9.78 pmol/L for CT-proET-1.

Outcomes

The primary end-point was a poor outcome, defined by a composite of the following criteria occurring within 30 days after inclusion: admission to an intensive care unit (ICU), non invasive ventilation, oro-tracheal intubation followed by mechanical ventilation), or death.

Secondary end-point was the etiology of the acute dyspnea episode, which was assessed by an expert panel (two independent experts, blinded to the results of studied biomarkers) after reviewing all information available on the medical files and from follow-up.

Study size

Based upon previous literature, we estimated the rate of the composite primary endpoint at 30-day to be 20%. With an hypothesis of a difference of 10% of the biomarker's area under the receiver operating curve (AUROC), it would have been necessary to include 150 patients. In order to allow a subgroup analysis for the three

main acute dyspnea causes (AECOPD, AHF, CAP) it would be therefore necessary to include 450 patients.

Statistical analysis

Data are expressed as mean ± standard deviation (SD) for Gaussian variables, median and 25 to 75% interquartile range for non-Gaussian variables, or number and percentage for nominal variables with 95% confident interval. Normality was assessed using the Kolmogorov-Smirnov test. Diagnostic variables (sensitivity, specificity, negative predictive value [NPV], positive predictive value [PPV]) and positive and negative likelihood ratio were calculated with their 95% confidence intervals (CI). Receiving operator characteristics (ROC) curves were constructed and their areas under the curves (AUROC) were calculated. Comparison of the two groups (poor vs good outcome) was performed using the Student t test, the Mann-Whitney U test, and Fisher's exact method when appropriate.

A multivariable analysis was performed using logistic regression to assess variables associated with the combined primary endpoint, and odds ratios (ORs) with their 95% CI were calculated. To avoid overfitting, a conservative approach was used and all variables associated with the primary endpoint with a p less than 0.1 were included, along with the five studied biomarkers (PCT, MR-proANP, MR-proADM, copeptin and pro-ET1). Correlation between all variables were calculated, and in case of a coefficient of correlation $R^2>0{\cdot}6$, only the most clinically significant variable was entered in the model. Calibration of the model was estimated with Hosmer-Lemeshow test, and discrimination with the c-index.

All analyses were performed using SPSS software (IBM, Armonk, NY), all P values were two-tailed and a P value of 0.05 was required to reject the null hypothesis.

Results

The flow-chart of the study is represented on figure 1. Among the 452 enrolled patients, 58 patients were excluded because major clinical data, blood samples, or follow-up were lacking Thus 394 patients were retained in the final analysis. Patients characteristics according to the group of severity (good and poor outcome), are reported in table 1. Mean age was 75± 15 year with 51% of male. The most frequent pre-existing chronic diseases were COPD, chronic heart failure and coronary arterial disease. Cough, crackling on auscultation and signs of right ventricular insufficiency were the most frequent signs on examination. One hundred and thirty seven patients (35%) had a poor outcome with 70 patients (18%) directly admitted from the ED to ICU, 58 (15%) deaths, and 15 patients secondarily admitted in ICU after initially admitted in medical wards (figure 1). The diagnoses were AHF for 183 patients (47%), CAP for 119 (30%), AECOPD for 90 (23%) and PE for 18 (5%). Patients with poor outcome had worse vital signs and blood gas parameters and more frequently experienced signs of respiratory distress on arrival (table 1).

Pro-hormone-type biomarkers and severe outcome risk-stratification

The values of PCT, MR-proADM, MR-proANP, CT-proET1 and copeptin in the good and poor outcome groups are reported in table 2. All pro-hormone-type biomarkers values but MR-proANP were significantly higher in the poor outcome group. The sensitivity, specificity, positive and negative likelihood ratio and AUROC for the combined primary endpoint and 30-day death are reported in table 3. All biomarkers but PCT exhibited high sensitivity (ranging from 0.83 to 0.98) but low specificity, for both severity endpoints. MR-proADM had the best sensitivity (0.95) in predicting the

combined endpoint but had a weak specificity, positive likelihood ratio and AUROC. The pro-hormone-type biomarkers performed better for 30-day death prediction with sensitivity ranging from 0.50 to 0.98 and AUROC ranging from 0.60 to 0.72 but exhibited low specificity (0.09 to 0.72).

The results of the logistic regression performed to assess variables associated with the combined primary endpoint are reported in table 4. The presence of either paradoxical abdominal breathing or cyanosis at admission was independently associated with poor outcome. When the pro-hormone-type biomarkers were added into the model, MR-proADM was the only one significantly associated with the combined primary endpoint (odd ratio 1.44 [95%CI: 1.13-1.82] p=0.003).

PCT and MR-proANP for the etiological diagnosis of severe acute dyspnea

In this cohort of severe acute dyspnea patients, we also sought to evaluate the diagnostic performances of PCT and MR-proANP to diagnose respectively CAP and AHF. For CAP diagnosis, PCT performances were: sensitivity 0.54, specificity 0.78, positive predictive value (PPV) 0.52, negative predictive value (NPV) 0.80, positive likelihood ratio (LR+) 2.49 and negative likelihood ratio (LR-) of 0.58. For AHF diagnosis, MR-proANP peformances were respectively: sensitivity 0.98, specificity 0.34, PPV 0.57, NPV 0.94, LR+ 1.48, LR- 0.06.

Discussion

Risk stratification for patients who present with acute severe dyspnea in the ED is crucial but often difficult, due to the lack of sensitivity and specificity of clinical examination, either associated to the arterial blood gas analysis (4). In this study we

conclude that the five tested pro-hormone-type biomarkers have a very low added value for the prediction of a composite endpoint of poor outcome.at 30-day

We selected for inclusion patients with severe acute dyspnea rather than any dyspneic patients attending the ED. This choice was deliberate because the risk stratification is even more difficult in patients who are clinically critical but without obvious criteria for mechanical ventilation or ICU admission. This is a major difference in patient selection in comparison with previous studies on biomarkers in dyspnea conducted in the ED (6,9,11). The severity of our population was assessed by an 18% rate of ICU admission and a 30-day mortality rate of 15%, much higher than those reported in similar cohorts of ED's dyspnea patients (10,11,17).

A number of pro-hormone-type biomarkers have been reported to have a good prognostic value in different etiologies of dyspnea: CAP for PCT, MR-proADM, copeptin and MR-proANP (13,21–23), AHF for MR-proANP and MR-proADM (10,24), AECOPD for MR-proADM and CT-proET1 (19). In 154 patients with dyspnea of uncertain origin in the ED, Cinar et al reported that MR-proADM had an OR of 8.5 and a 0.81 AUROC for 30-day mortality with a threshold of 1.5 nmol/L (17). In a multicenter study on 441 acute dyspnea episodes in the ED, Travaglino et al reported that PCT and MR-proADM had an AUROC of respectively 0.70 and 0.62 for 30-day mortality (11). Other authors have reported the long-term prognostic value (1 to 4 year-mortality prediction) of MR-proADM and MR-proANP measured at the initial ED visit, although the practical usefulness of such information is uncertain (6,9). All these studies included unselected patients with shortness of breath, regardless of the initial severity.

In our multicenter study, we focused on a subpopulation of 394 dyspneic patients attending the ED with already severity criteria (see inclusion criteria), and found that

pro-hormone-type biomarkers had limited added value for the risk stratification. Most of these biomarkers were very sensitive and exhibited higher serum values in the group of patients who would experience poor outcome, but they were poorly specific (see table 3). MR-proADM was the only independent variable significantly associated with the combined end point (ICU admission-invasive ventilation-death) with an OR of 1.43 [1.13-1.82]. This is in accordance with previous studies identifying MR-proADM as a stress biomarker among dyspneic patients (6,9,11,17). However, the MR-proADM concentration was less informative than the two clinical criteria: paradoxical abdominal breathing and cyanosis with respectively OR of 2.48 [1.31-4.68] and 3.18 [1.46-6.89]. Because these clinical criteria may be under-recognized and/or missed by the physician, an elevated MR-proADM concentration could be useful to warn about the potentially critical state of the patient.

On an other hand, we confirm that in a cohort of severe acute dyspnea patients, PCT and MR-proANP may be useful for the physician in helping the identification of the main cause of the dyspnea episode, respectively CAP and AHF. For the diagnosis of CAP, PCT showed a PPV and NPV of 52% and 80% respectively, while MR-proANP had a 57% PPV and 94% NPV for the diagnosis of AHF. The diagnostic characteristics were very similar to previous studies and point out that both biomarkers may be used rather with a view of rule-out than of rule-in in the population of severe acute dyspnea patients (10,15,17,25).

Our study has several limitations. First, although the cohort comprised 394 patients, a subgroup analysis for each main etiology of acute dyspnea was not possible due to a lack of power. Second, as we were interested in severe acute dyspnea patients, our results may not be extrapolated to all the dyspnea patients attending the EDs. Third, this study was mainly negative and thus its power to detect a significant added value

of biomarkers is important. However, we appropriately defined the hypothesis tested and think that if a significant added value could have been detected by a larger study it would have been of very limited clinical significance.

In summary,, for patients with severe acute dyspnea as the main complaint when entering the ED, clinical severity criteria are better predictors of poor outcome than pro-hormone type biomarkers which have little added-value. Among them, MR-proADM has the best added value for the risk stratification. We confirm that MR-proANP and PCT may be helpful for the identification of the main cause of dyspnea.

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Table 1: Characteristics of study cohort.

	All patients		Good Outcome		Poor Outcome		P value
	N=394	%	N=257	65%	N=137	35%	
Age	75 (15)						
Male sex	199	51%	124	48%	75	55%	0.20
Living at home	300	76%	193	75%	107	78%	0.50
Past medical history							
Smoker	113	29%	67	26%	46	34%	0.11
Hypertension	219	55%	145	56%	74	54%	0.60
Diabete mellitus	79	20%	48	19%	31	23%	0.40
Chronic alcohol intake	27	7%	13	5%	14	10%	0.05
Admitted to hospital within 6 months	126	32%	75	29%	51	37%	0.10
Chronic respiratory disease	167	43%	112	44%	55	40%	0.50
COPD	94	24%	61	24%	33	24%	0.90
Asthma	29	7%	21	8%	8	6%	0.50
other	44	11%	30	12%	14	10%	0.80
Cardiovascular disease	259	65%	169	66%	90	66%	1.00
Chronic Heart failure	116	29%	77	30%	39	29%	0.80
coronary arterial disease	109	28%	71	28%	38	28%	1.00
Other chronic diseases							
Cerebro-vascular disease	40	10%	31	12%	9	7%	0.09
Dementia	31	8%	16	6%	15	11%	0.10
Parkinson disease	9	2%	7	3%	2	2%	0.50
Chronic kidney disease	38	10%	26	10%	12	9%	0.70
Cancer	63	16%	41	16%	22	16%	1.00
Vital parameters on arrival							
Heart rate bpm (SD)	95	(23)	94	(22)	99	(25)	0.03
Respiratory rate cycle/mn [IQR]	28	[25 ; 34]	28	[24 ; 32]	32	[27 ; 35]	<0.001
Systolic Blood Pressure mmHg (SD)	138	(30)	140	(30)	134	(31)	0.10
Pulse oxymetry % [IQR]	90	[86 ; 93]	92	[88 ; 94]	88	[83 ; 92]	<0.001
Temperature °C (SD)	37.2	(1.2)	37,2	(1.0)	37,1	(1.1)	0.30
Clinical Examination							
Sweating	40	10%	18	7%	22	16%	<0.01
Mottling	26	7%	9	4%	17	12%	<0.001
Cough	148	38%	103	40%	45	33%	0.16
paradoxical abdominal respiration	65	17%	27	11%	38	28%	<0.0001
Cyanosis	44	11%	15	6%	29	21%	<0.0001
Wheezing	114	29%	81	32%	33	24%	0.12
Crackling	237	60%	182	59%	85	62%	0.60

Ronchi	88	22%	58	23%	30	22%	0.90
Chest pain	51	13%	33	13%	18	13%	0.90
Peripheral right heart insufficiency signs	168	43%	111	43%	57	42%	0.80
Chest X-ray							
Cardiomegaly	108	37%	62	33%	46	45%	0.06
Pleural effusion	53	18%	30	16%	23	22%	0.20
Consolidation	65	22%	40	21%	25	24%	0.60
Laboratory results							
Hemoglobin $g.dl^{-1}$ (SD)	12.9	(1.9)	12.9	(1.9)	12.9	(2.0)	0.30
White cell count $Giga.l^{-1}$ (SD)	11.3	(6.9)	11.3	(7.4)	11.4	(5.8)	0.80
Neutrophiles $Giga.l^{-1}$ (SD)	9.1	(5.9)	8.6	(5.0)	10.1	(7.2)	0.04
Platelets $Giga.l^{-1}$ (SD)	247	(110)	250	(106)	240	(118)	0.40
Arterial pH (SD)	7.39	(0.08)	7.41	(0.07)	7.36	(0.1)	<0.001
PCO2 mmHg [IQR]	40	[34 ; 48]	38	[34 ; 45]	43	[36 ; 61]	<0.001
PO2 mmHg [IQR]	67	[59 ; 84]	68	[61 ; 84]	66	[55 ; 82]	0.04
HCO3 $mmol.\ l^{-1}$ (SD)	25.2	(5.4)	24.6	(4.2)	26.3	(6.8)	<0.01
SaO2 % [IQR]	93	[90 - 96]	94	[91 ; 97]	92	[86 ; 96]	<0.01
Creatinine $\mu mol.\ l^{-1}$ [IQR]	88	[66 ; 124]	86	[65 ; 116]	94	[69 ; 129]	0.09

SD, standard deviation; IQR, 25-75% interquartile range COPD: chronic obstructive pulmonary disease

Table 2: PCT, MR-proADM, MR-proANP, pro-ET1 and copeptin values at admission according to the outcome (good prognosis and the combined severe outcome endpoint). Data are expressed as median and their 25 to 75% interquartile ranges

	All patients N=394	Good Outcome N=257	Poor Outcome N=137	P value
MR Pro ADM (nmol/L)	1.3 [0.88 ; 1.96]	1.25 [0.77 ; 1.82]	1.47 [1.02 ; 2.71]	<0.001
CT-Pro ET 1	108 [74 ; 160]	99 [71 ; 142]	128 [80 ; 189]	<0.001
Copeptine (pmol/L)	44 [16 ; 105]	33 [14 ; 76]	71 [28 ; 185]	<0.001
MR-pro ANP (pmol/L)	251 [114 ; 470]	238 [101 ; 442]	282 [142 ; 583]	0.05
PCT (µg/L)	0.13 [0.08 ; 0.35]	0.13 [0.07 ; 0.28]	0.16 [0.1 ; 0.6]	0.01

procalcitonin (PCT), midregional pro-A-Type natriuretic (MR-proANP), midregional pro-adrenomedullin (MR-proADM), pro-vasopressin (copeptin) and pro-endothelin1 (CT-pro ET1)

Table 3A: Prediction of a poor outcome by pro-hormone-type biomarkers at admission. Data are expressed with their [95% confidence interval]. Poor outcome was defined by a composite of the following criteria occurring within 30 days after inclusion: admission to an intensive care unit (ICU), non invasive ventilation, oro-tracheal intubation followed by mechanical ventilation), or death.

Biomarker (threshold)	Sensitivity	Specificity	LR+	LR-	AUROC
MR Pro ADM (0.55 nmol/L)	0.95 [0.90 - 0.98]	0.09 [0.06 - 0.14]	1.05 [0.99 - 1.11]	0.48 [0.18 - 1.09]	0.61 [0.54 - 0.67]
CT-Pro ET 1 (66.6 pmol/L)	0.86 [0.79 - 0.91]	0.22 [0.17 - 0.28]	1.10 [1.00 - 1.21]	0.64 [0.39 - 1.01]	0.49 [0.44 - 0.55]
Copeptine (17.4 pmol/L)	0.83 [0.75 - 0.89]	0.33 [0.28 - 0.40]	1.25 [1.11 - 1.40]	0.80 [0.33 - 0.75]	0.59 [0.52 - 0.65]
MR pro ANP (85.2 pmol/L)	0.86 [0.79 - 0.91]	0.21 [0.17 - 0.27]	1.09 [0.99 - 1.20]	0.65 [0.40 - 1.04]	0.55 [0.49 - 0.61]
PCT (0.25 mmol/L)	0.39 [0.31 - 0.48]	0.73 [0.67 - 0.78]	1.47 [1.09 - 1.97]	0.83 [0.78 - 0.96]	0.55 [0.48 - 0.62]

LR+: positive likelihood ratio; LR-: negative likelihood ratio, AUROC: area under the receiving operator curve. procalcitonin (PCT), midregional pro-A-Type natriuretic (MR-proANP), midregional pro-adrenomedullin (MR-proADM), pro-vasopressin (copeptin) and pro-endothelin1 (CT-pro ET1)

Table 3B: Prediction of mortality at 30-day by the pro-hormone-type biomarkers at admission. Data are expressed with their [95% confidence interval]. LR+: positive likelihood ratio; LR-: negative likelihood ratio

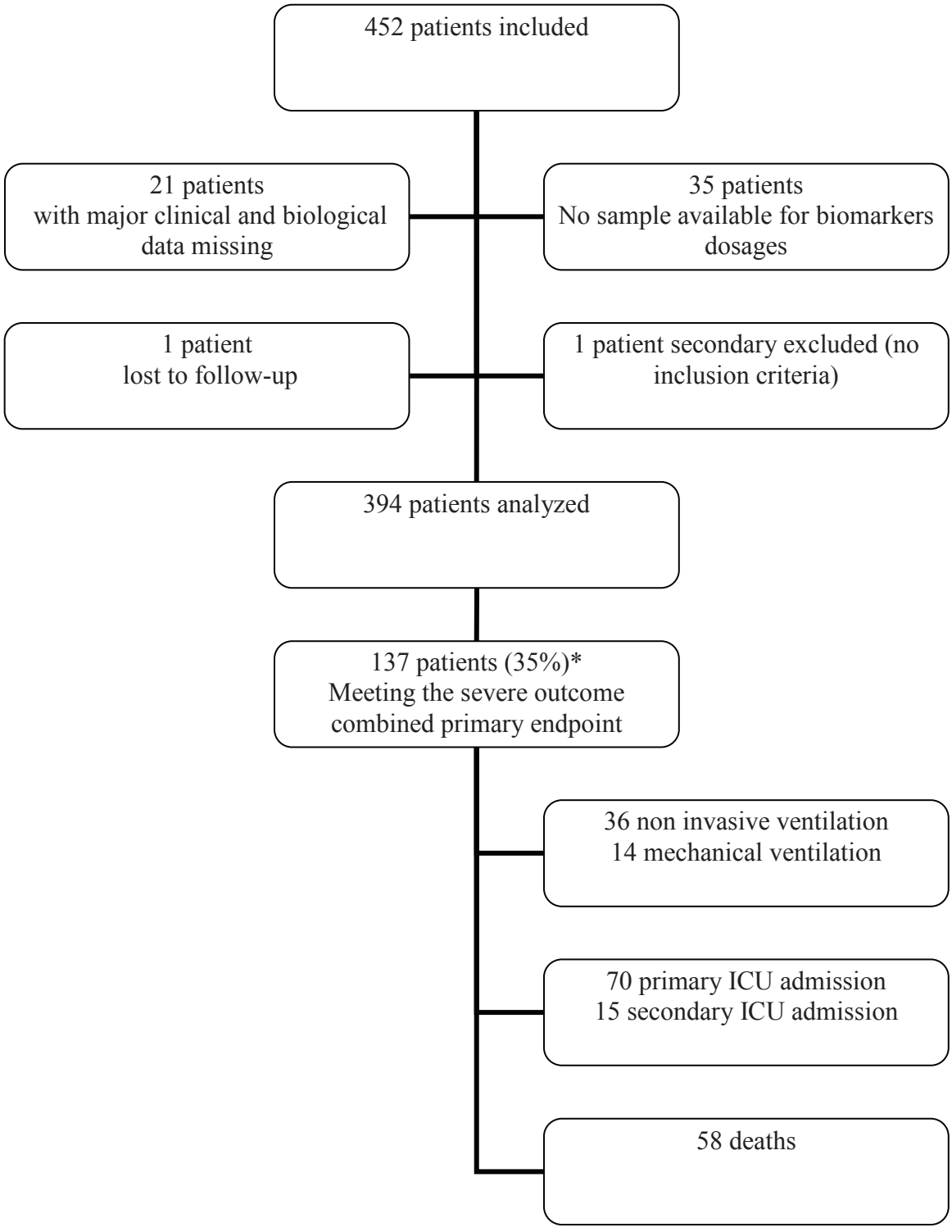
Biomarker (threshold)	Sensitivity	Specificity	LR+	LR-	AUROC
MR Pro ADM (0.55 nmol/L)	0.98 [0.89 - 0.99]	0.09 [0.06 - 0.12]	1.07 [1.00 - 1.13]	0.20[0.01 - 1.00]	0.69 [0.60- 0.77]
CT-Pro ET 1 (66.6 pmol/L)	0.91 [0.80 - 0.97]	0.21 [0.17 - 0.26]	1.16 [1.03 - 1.26]	0.42 [0.16 - 0.90]	0.70 [0.61 - 0.78]
Copeptine (17.4 pmol/L)	0.91 [0.80 - 0.97]	0.31 [0.26 - 0.36]	1.32 [1.16 - 1.46]	0.28 [0.11 - 0.60]	0.72 [0.64 - 0.78]
MR pro ANP (85.2 pmol/L)	0.93 [0.82 - 0.98]	0.21 [0.17 - 0.26]	1.18 [1.05 - 1.30]	0.33 [0.11 - 0.79]	0.68 [0.60 - 0.75]
PCT (0.25 mmol/L)	0.50 [0.37 - 0.63]	0.72 [0.67 - 0.77]	1.78 [1.28 - 2.40]	0.69 [0.51 – 0.88]	0.60 [0.51 - 0.68]

LR+: positive likelihood ratio; LR-: negative likelihood ratio, AUROC: area under the receiving operator curve. procalcitonin (PCT), midregional pro-A-Type natriuretic (MR-proANP), midregional pro-adrenomedullin (MR-proADM), pro-vasopressin (copeptin) and pro-endothelin1 (CT-pro ET1)

Table 4: Multivariable analysis to assess variables associated with a poor outcome

Variable	P value	Odds ratios [95%CI]
Heart rate	0.72	1.00 [0.99-1.01]
Sweeting	0.16	1.80 [0.79-4.06]
Mottling	0.45	1.48 [0.53-4.12]
Paradoxical abdominal respiration	<0.005	2.48 [1.31-4.68]
Chronic alcohol intake	0.49	1.43 [0.52-3.95]
Cerebro-vascular disease	0.15	1.90 [0.80-4.55]
Cyanosis	0.003	3.18 [1.46-6.89]
MR-proADM	0.003	1.43 [1.13-1.82]
Pro-ET1	0.68	1.00 [0.99-1.00]
Copeptine	0.93	1.00 [0.99-1.00]
MR-proANP	0.49	1.00 [0.99-1.00]
PCT	0.28	0.97 [0.92-1.02]

CI: confidence interval. Procalcitonin (PCT), midregional pro-A-Type natriuretic (MR-proANP), midregional pro-adrenomedullin (MR-proADM), pro-vasopressin (copeptin) and pro-endothelin1 (CT-pro ET1)



* a patient could have more than one severe outcome combined endpoint

Figure 1: flow chart of the study. ICU: intensive care unit.

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Appendice 3 : Etude hFABP

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Original Contribution

Heart-type fatty acid binding protein and the diagnosis of acute coronary syndrome in the ED☆

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Abstract

Background: In combination with cardiac troponin, heart-type fatty acid binding protein (h-FABP)—a biomarker of myocardial necrosis—offers the possibility of rapidly eliminating the diagnosis of acute myocardial infarction (AMI).

Objective: The main objective of this study was to assess the incremental value of h-FABP to cardiac troponin for a rapid elimination of AMI, according to the pretest probability (PTP) of AMI.

Methods: In consecutive patients presenting to emergency departments (ED) with chest pain less than 6 hours suggestive of AMI, h-FABP levels were measured, blinded to the ED physicians, who were asked to quote the PTP of AMI. The discharge diagnosis was adjudicated by 2 independent experts, blind to the h-FABP level.

Results: Three hundred seventeen patients (mean age of 57 years) were included in whom 149 had (47%) low, 117 (37%) moderate, and 51 (16%) high PTP. The final diagnosis was AMI in 45 patients (14%), including 16 STEMIs (5%). The negative predictive value for diagnostic elimination of AMI of an h-FABP less than 3 μg/L, combined with a negative cTnI was not higher than that of cardiac troponin I (cTnI) alone (96% [95% confidence interval, 93%-98%] vs 95% [93%-98%]), regardless of the PTP). Even in the low-PTP group, we did not demonstrate a significant improvement in negative predictive value with the addition of h-FABP, compare with that of cTnI alone (100% [97%-100%] vs 99% [96%-100%]).

☆ Conflicts of interest: CCG, SG, BR, and PR received honoraria from B.R.A.H.M.S; PR received honoraria from bioMérieux, RocheDiagnostics, BMD.

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Conclusion: In triage of patients with chest pain, use of h-FABP does not provide useful additional information to cTnI for excluding the diagnosis of ST-elevation myocardial infarction and non–ST-elevation myocardial infarction diagnosis, whatever the PTP.

1. Introduction

Early detection of acute coronary syndromes (ACSs) remains suboptimal and a major concern in the field of emergency medicine. Patients with chest pain represent approximately 15 million consultations per year in the US emergency departments (EDs) [1,2]. Although quite specific [3], electrocardiographic (ECG) ST elevation has only a 50% to 60% sensitivity for the diagnosis of myocardial necrosis [4]. In emergency patients with chest pain, cardiac troponins (cTns) do not reliably exclude non–ST-elevation myocardial infarction (NSTEMI) without repeated negative measurements over 4 to 6 hours [1]. Therefore, there is a need for a fast and reliable test to facilitate triage, diagnosis, and adequate treatment strategies. This is particularly important in patients presenting with an NSTEMI or atypical symptoms and/or noncontributive ECG.

The heart-type fatty acid binding protein (h-FABP) is a biomarker of myocardial necrosis and injury that offers several theoretical advantages over cTn. Heart-type fatty acid binding protein is a 15-kd soluble protein, which is a powerful regulator of the mitochondrial β-oxidative system. It represents 10% of the whole cardiomyocytes cytosolic proteins [5]; is undetectable in normal conditions; but is released from the myocardium under various types of injury, including myocardial ischemia [6]. Owing to its small size, h-FABP is released quickly into the circulation when membrane integrity is compromised in response to myocardial injury. Levels of h-FABP are detectable as early as 2 to 3 hours and typically return to baseline levels within 12 to 24 hours after the initial insult [7]. Consistent with these findings, several studies have shown that h-FABP is a sensitive marker for the diagnosis of NSTEMI [8] and might be more sensitive than conventional cTn assays when measured soon after the early onset of symptoms even in the prehospital setting [9]. However, previous studies have not demonstrated any diagnostic value of this biomarker in other settings [10]. Use of h-FABP has been also restricted to clinical research because of the lack of a fast and easy-to-use test. However, a novel 1-step qualitative assay for the detection of h-FABP has recently been developed, the CardioDetect assay (Rennesens GmbH, Berlin, Germany; distributed by BMD, Buc, France), which allows diagnosis of acute myocardial infarction (AMI) within 30 minutes of chest pain [11].

Our main objective in this study was to assess whether this assay provides additional diagnostic value to that of the conventional cTn in ruling out ST-elevation myocardial infarction (STEMI) and NSTEMI in patients presenting to the ED with chest pain, according to their pretest probability (PTP).

2. Patients and methods

2.1. Study population and design

During the period from August 2005 to January 2007 in 3 hospitals affiliated to University of Paris, we prospectively enrolled consecutive out-hospital patients (>18 years) presenting to the ED with symptoms suggestive of AMI such as chest pain indicative of ACS and angina pectoris with onset or peak within the previous 6 hours. Patients with terminal kidney failure requiring dialysis were excluded. The study was performed according to the principles of the Declaration of Helsinki and approved by the local ethics committee (Comité de Protection des Personnes Ile-de-France VI, CHU Pitié-Salpétrière Hospital, Paris, France). Because routine medical care was unchanged, waived inform consent was authorized. We followed the recommendations concerning the reporting of diagnostic studies, the Standards for Reporting of Diagnostic Accuracy initiative [12] and evaluation of a biomarker [13].

2.2. Routine clinical assessment

As part of the routine assessment in our institutions, all patients underwent an initial clinical evaluation that included clinical history, physical examination, 12-lead ECG, pulse oximetry, routine blood tests, and chest x-ray. After these routine tests were done and before cardiac biomarker results were available (thus, before cardiac troponin I [cTnI] levels), emergency physicians were asked to assign an "empirical" clinical probability of AMI to each case (a low, medium, or high probability) [14].

Cardiac troponin I was measured at presentation and repeated after 3 to 9 hours, for as long as was clinically indicated. Then, according to the diagnosis of NSTEMI or STEMI, the patients were admitted directly to the coronary care unit (CCU) for further evaluation and treatment or directly to the catheterization laboratory for primary percutaneous coronary intervention. However, the timing and treatment of patients were left to the discretion of the attending physicians according to the suspected diagnosis. Emergency physicians in charge were blinded to the results of h-FABP, and chemical pathologists were blinded to the emergency diagnosis suspected by the physicians.

2.3. Adjudicated final diagnosis

To determine the causal diagnosis for each patient, 2 independent experts (emergency physicians) blinded to

the results of h-FABP reviewed all available medical records (including patient history, physical findings, results of laboratory and radiologic testing, ECG, echocardiography, cardiac exercise test, coronary angiography, and summary chart at discharge) pertaining to the patient from the time of ED presentation to 30-day follow-up. If there was diagnostic disagreement, cases were reviewed and adjudicated in conjunction with a third expert (also an emergency physician).

Myocardial necrosis (ie, STEMI and NSTEMI) was defined according to the joint European Society of Cardiology/American College of Cardiolology/American Heart Association/World Heart Federation Task Force redefinition of myocardial infarction (MI) guidelines [15]. Diagnosis of myocardial necrosis was made when there was evidence of cTnI increase (above the 99th percentile of the upper reference limit) in association with at least one of the following: ECG ST-T changes or new Q wave, images of new lost viable myocardium, symptoms of ischemia, or a normal cTnI on admission. Unstable angina was diagnosed (1) in patients with normal cTnI levels and typical angina at rest, (2) a sudden increase in episodes of a previously stable angina, (3) according to results of cardiac exercise testing or cardiac catheterization stated in the summary chart, and (4) in ambiguous cases in which follow-up information revealed a relapse of myocardial necrosis or a sudden unexpected cardiac death within 30 days.

3. Biochemical analysis

3.1. Cardiac troponin I measurements

In 2 EDs (Hôpital Cochin and La Pitié Salpêtrière Hospital), plasma cTnI concentrations were routinely measured on an X-pand HM analyzer, using the cTnI immunoassay (Siemens Healthcare Diagnostics, Inc, Newark, NJ). This 1-step enzyme immunoassay based on the "sandwich" principle requires 50 μL of sample and uses 2 mouse monoclonal antibodies. After incubation, the bound fraction is separated using antibody-coated chromium dioxide microparticles and quantified by enzymocolorimetry. The measuring range extends from 0.04 to 40.00 μg/L. The 99th percentile for this method is 0.07 μg/L, with coefficients of variations (CVs) between 15% and 22%; the limit of quantitation (functional sensitivity, ie, the lowest analyte concentration that can be reproducibly measured with a between-run CV of $\leq$10%) is 0.14 μg/L.

In the Hôpital Bicêtre, plasma cTnI concentrations were routinely measured on an Access analyser (Beckman Coulter, Inc, Brea, CA). The measuring range of this 1-step chemiluminescent immunoassay extended from 0.01 to 100.00 μg/L. The 99th percentile for this method is 0.04 μg/L, and the CV 10% according to the manufacturer is 0.06 μg/L.

3.2. h-FABP measurement

Patients were tested with the CardioDetect assay (Rennesens GmbH, Berlin, Germany; distributed by BMD, Buc, France). This is a 1-step semiquantitative h-FABP test, which is a rapid chromatographic immunoassay designed for determination of soluble h-FABP in whole blood or plasma samples. The test, as previously described [16,17], was performed in our laboratory applying 100 μL of a plasma sample of each patient on to the test strip. After 15 minutes, the test was read by 2 independent readers. If the sample contained h-FABP with a concentration below the detection limit (<3 μg/L), only the control band at the control zone was read (negative test). The test was recorded as positive if there was presence of a band at the test zone, in addition to the control band.

When there was disagreement, a third independent expert was called for a final decision. All tests were also scanned using a CardioDetect quant instrument (Rennesens GmbH, Berlin, Germany) for quantitative interpretation of the results, and these quantitative results were used for receiver operating characteristic (ROC) analysis. It should be noted from the outset that we observed discrepancies between qualitative and quantitative results. Thirty-nine patients with positive h-FABP test were shown as having less than 3 ng/L by the CardioDetect quant.

3.3. Calculation of the estimated glomerular filtration rate

Estimated glomerular filtration rate (eGFR) values (in milliliters per minute per 1.73 m^2) were calculated using the revised [18] Modification of Diet in Renal Disease formula [19]: eGFR = 175$*$[serum creatinine (mg/dL) $-$ 1.154]$*$[age (years) $-$ 0.203]. The values thus calculated were then multiplied by 0.742 for women. Estimated glomerular filtration rate values less than 60 mL/min per 1.73 m^2 were indicative of kidney dysfunction.

3.4. Statistical analysis

Continuous variables are presented as mean $\pm$ SD or median (with interquartile range) for non–Gaussian-distributed variables; categorical variables, as numbers and percentages. Normality was assessed using the Kolmogorov-Smirnov test. Continuous variables were compared with the Mann-Whitney U test or the Student t test, as indicated, and categorical variables, using the Pearson χ^2 test. Correlations among continuous variables were assessed with the use of the Spearman rank-correlation coefficient. Logistic regression was used to combine cTn and h-FABP in the diagnosis of AMI and to adjust for other baseline variables. Receiver-operating characteristic curves were constructed to compare the ability of cTn and h-FABP to diagnose AMI. Comparison of areas under the ROC curves (AUC) was performed as

recommended [13]. All hypothesis testing were 2 tailed, and $P < .05$ was considered significant. Statistical analysis was performed using StatView for Windows (version 5.0; SAS Institute, Cary, NC) and MedCalc for ROC analysis (Medcalc software, Mariarkerke, Belgium). Graphs were built with GraphPad Prism 5 (Graphpad software, Inc, La Jolla, CA).

4. Results

4.1. Patient characteristics

Over 18 months, 317 consecutive patients were enrolled. Baseline characteristics of patients according to their empirical probability of ACS are shown in Table 1. Mean age was 57 ± 17 years, and 205 (65%) were male. As expected in this unselected emergency population, there was a significant proportion of elderly patients (30%, ie, 96 patients were 65 years and older) and patients with a prior history of myocardial ischemia (26%, n = 83). Chest pain was considered typical of ACS in 43% (n = 136) of patients. The adjudicated final diagnosis was AMI in 14% of patients (n = 45), unstable angina in 3% (n = 11), and other diagnoses 82% (n = 261). Of the patients with AMI, 27% (n = 12) were diagnosed having STEMI and 73% (n = 33) as having NSTEMI. According to the PTP group, AMI (ie, NSTEMI and STEMI) was diagnosed in 3% of low, 16% moderate, and 39% high PTP. At 30 days, there were 3 deaths (2 in the MI group and 1 in the other cause group) and 4 relapses of ACS in the AMI group. Eighteen percent (n = 6) of patients with NSTEMI had a negative initial cTnI. In this patient subgroup, 2 patients were found to have a positive h-FABP test. Thus, 4 patients (12%) with final

Table 1 Baseline characteristics of the population according to PTP

	All patients	PTP of ACS			
		Low	Moderate	High	*P* *
N	317	149	117	51	
Percentage of all patients	100	47	37	16	
Age (y)	57 ± 17	53 ± 18	61 ± 16	60 ± 18	.001
Men	205 (65)	88 (59)	85 (73)	32 (63)	.067
Systolic BP (mm Hg)	141 ± 28	135 ± 24	147 ± 30	147 ± 30	.001
Diastolic BP (mm Hg)	80 ± 16	78 ± 15	83 ±8	83 ± 15	.047
Cardiac rate	85 ± 45	86 ± 23	82 ± 22	78 ± 18	.126
Spo_2 (%)	97 ± 3	97 ± 4	97 ± 2	97 ± 2	.639
Familial history of CAD	100 (32)	26 (17)	54 (46)	20 (39)	<.0001
Personal history of CAD	83 (26)	12 (8)	46 (39)	25 (49)	<.0001
Dyslipidemia	113 (36)	28 (19)	58 (50)	27 (53)	<.0001
Smoking	128 (40)	50 (34)	52 (44)	26 (51)	.05
Diabetes	44 (14)	9 (6)	23 (20)	12 (24)	.0006
Hypertension	116 (37)	35 (23)	55 (47)	26 (51)	<.0001
History of heart failure	21 (7)	4 (3)	10 (9)	7 (14)	.014
Typical thoracic pain	136 (43)	56 (38)	55 (47)	25 (49)	.176
Coronarography	83 (26)	20 (13)	37 (32)	26 (51)	<.0001
Treatment received during the first 24 h after admission					
Aspirin	119 (38)	27 (18)	59 (50)	33 (65)	<.0001
Clopidogrel	54 (17)	7 (5)	26 (22)	21 (41)	<.0001
LMWH	68 (21)	14 (9)	33 (28)	21 (41)	<.0001
Anti-GPIIb/IIIa	3 (1)	1 (1)	0 (0)	2 (4)	.048
Hospital admission	194 (61)	67 (45)	81 (69)	44 (86)	<.0001
Admission in CCU	138 (44)	38 (26)	60 (51)	40 (78)	<.0001
Patients with positive cTnI at admission	40 (13)	7 (5)	19 (16)	14 (27)	<.0001
eGFR (mL/min per $1.73m^2$)	77 (62-94)	81 (67-101)	74 (62-92)	75 (57-87)	.017
Final diagnosis of AMI	45 (14)	5 (3)	25 (21)	15 (29)	<.0001
STEMI	16 (5)	2 (1)	9 (8)	5 (10)	.015
NSTEMI	29 (9)	3 (2)	16 (14)	10 (20)	
Final diagnosis of UA	11 (3)	0 (0)	4 (3)	7 (14)	<.0001
Other diagnosis [a]	261 (82)	144 (97)	88 (75)	29 (57)	<.0001

BP indicates blood pressure; CAD, coronary acute disease; LMWH, low-molecular-weight heparin; ICU, intensive car unit; UA, unstable angina. Results are in mean ± SD, median (25th-75th percentile), or number (percentage).

[a] Including stable angina (n = 63), pulmonary embolism (n = 16), myopericarditis (n = 43), heart failure (n = 5), and others.

* Between AMI vs others (unstable angina and other diagnosis).

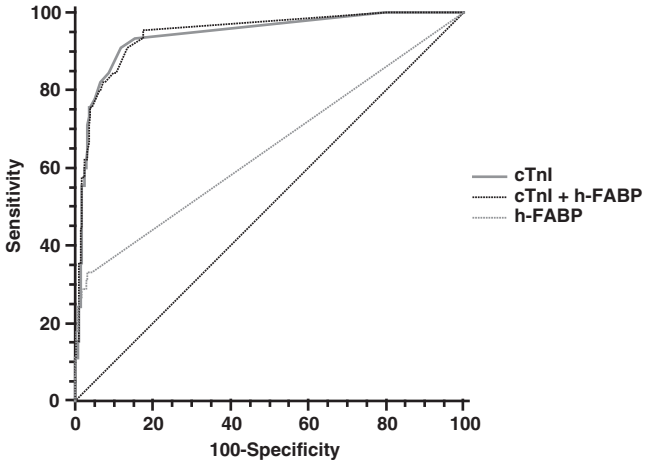

Fig. 1 Receiver operating characteristic curves for the diagnosis of AMI (STEMI and NSTEMI). For this analysis, h-FABP results less than 3 μg/L (negative tests) were considered as 3 μg/L; cTnI and h-FABP values were log transformed before association.

diagnosis of NSTEMI remained with both negative cTnI and h-FABP at admission.

The highest AUC for the diagnosis of myocardial necrosis was for initial cTnI (AUC, 0.94 [95% confidence interval {CI}, 0.91-0.96] vs 0.65 [95% CI, 0.55-0.77] for h-FABP) (P = .001) as seen in Fig. 1. The AUC was not significantly improved when h-FABP was associated with cTnI: 0.94 (95% CI, 0.91-0.97) vs 0.94 (0.91-0.96) for cTnI alone (P = .54). The optimal cutoff point for h-FABP given by the ROC analysis was 3.3 μg/L (sensitivity 33.3% [20%-49%], specificity 96.7% [94%-99%]). The sensitivities and specificities of different cardiac markers, alone or in association, are reported in Table 2A. Cardiac troponin I, alone or in combination with h-FABP, had a comparable negative predictive value (NPV), respectively, of 95% (93%-98%) vs 96% (93%-98%) in all patients and 99% (96%-100%) vs 100% (97%-100%) in low-PTP patients. The same results were noted when considering only chest pain of less than 3 hours and chest pain of more than 3 hours, as seen in Table 2B.

It should be noted that in all subgroups, specificity and positive predictive value were significantly worsened when cTnI was combined with h-FABP, as compared with cTnI alone.

5. Discussion

In our multicenter study, we were unable to demonstrate any incremental value of h-FABP to cTnI for the diagnosis of myocardial necrosis.

Several studies have evaluated h-FABP in patients with chest pain. However, various settings (cardiology units, ED, or prehospital) have reached conflicting conclusions.

Table 2 Diagnostic performances for the diagnosis of AMI (STEMI and NSTEMI)

A.	Se	Spe	PPV	NPV
In all patients (n = 317)				
Positive cTnI *	71 (55-83)	97 (94-98)	78 (62-89)	95 (92-97)
Positive h-FABP$	62 (47-76)	86 (82-90) †	43 (31-56) †	93 (89-96)
Positive cTnI * and/or h-FABP$	80 (65-90)	85 (80-87) †	46 (35-58) *	96 (93-98)
In low-PTP group (n = 148)				
Positive cTnI *	75 (22-99)	98 (94-100)	57 (20-88)	99 (96-100)
Positive h-FABP$	75 (22-99)	92 (87-96)	27 (9-55)	99 (95-100)
Positive cTnI * and/or h-FABP$	100 (40-100)	92 (86-96) ‡	29 (11-56)	100 (97-100)
B.	**Se**	**Spe**	**PPV**	**NPV**
Chest pain onset <3 h (n = 193)				
Positive cTnI *	71 (50-87)	96 (91-98)	72 (50-87)	96 (91-98)
Positive h-FABP$	63 (41-80)	86 (80-91) ‖	40 (25-57) ‖	94 (89-96)
Positive cTnI * and/or h-FABP$	75 (53-89)	85 (78-90) §	41 (27-57) ‖	96 (91-98)
Chest pain onset ≥3 h (n = 75):				
Positive cTnI *	60 (27-86)	100 (93-100)	100 (52-100)	94 (85-98)
Positive h-FABP$	40 (14-73)	85 (73-92) ¶	29 (10-58) ¶	90 (79-96)
Positive cTnI * and/or h-FABP$	70 (35-92)	85 (73-92) ¶	41 (19-67) ¶	95 (85-99)

Se indicates sensitivity; Spe, specificity; PPV, positive predictive value. Values are expressed as a percentage.

* P < .05 vs positive cTnI in all patients.

† P < .001 vs positive cTnI in all patients.

‡ P < .05 vs positive cTnI in low PTP group.

‖ P < .05 vs positive cTnI in chest pain onset less than 3 hours.

§ P < .001 vs positive cTnI in chest pain onset less than 3 hours.

¶ P < .05 vs positive cTnI in chest pain onset 3 hours or greater.

In a preliminary study with a high prevalence of STEMI, Ecollan et al [9] reported a higher sensitivity (87% vs 22% for cTn alone) of h-FABP for the diagnosis of AMI in 108 consecutive patients who presented less than 3 hours after the onset of symptoms and in whom the first medical care was delivered by a mobile intensive care unit. Similarly, Liao et al [20] included 74 patients who presented within 2.2 hours after the onset of chest pain, among whom 54 (73%) had confirmed AMI. At presentation, h-FABP gave the highest sensitivity of 83.3%. In addition, myocardial necrosis could be identified significantly earlier by h-FABP than cTnI (17 vs 6 patients; $P < .05$). Unfortunately, in these studies, the test was not performed blinded to the physicians in charge, leading to possible bias in the interpretation of results. Haltern et al [8] prospectively enrolled 97 emergency patients with acute ischemic–type chest pain and demonstrated a greater sensitivity of h-FABP in the first 4 hours of symptoms (86% vs 42% for cardiac troponin T [cTnT]; $P < .05$). Although combining h-FABP and cTnT improved the sensitivity in the diagnosis of AMI (97% vs 71%; $P < .05$), they also demonstrated a greater misclassification rate (25% vs 9%; $P < .05$).

McCann et al [21] enrolled 415 patients presenting to 2 CCUs within 24 hours of onset of acute ischemic–type chest pain, in whom 48% had a final diagnosis of AMI. In patients presenting less than 4 hours after the onset of symptoms, the sensitivity of h-FABP for MI was significantly higher than the cTnT measured at (73% vs 55%; $P = .043$). However, their results may not necessarily be applicable to lower risk populations, such as all patients with chest pain presenting at an ED. Recently, Charpentier et al [10] published the largest single-center study on h-FABP and ischemia-modified albumin for the detection of early AMI. They included 677 emergency patients who presented within 12 hours of the last episode of chest pain. Their results suggested that neither ischemia-modified albumin nor h-FABP was accurate biomarker for early diagnosis of ACS. Heart-FABP was predictive of the diagnosis of ACS (odds ratio, 4.65; 95% CI, 2.39-9.04) with a specificity at 97% and sensitivity at 14%. However, h-FABP did not add significant additional information to a predictive model that included the usual diagnostic tools for the management of non–ST-elevation ACS ($P = .40$). However, their end point criterion was the diagnosis of ACS, not AMI. The conflicting results between all these studies and that reported here can be explain by the setting, the prevalence of the diseases (AMI or ACS), and the delay between the onset of chest pain and the method of measurement for h-FABP. Thus, our study used a method close to that recommended by the Standards for Reporting of Diagnostic Accuracy statement for reporting studies on diagnostic accuracy [12], and the evaluation of h-FABP in relation to PTP. It should be noted that none of the previous studies evaluated the diagnostic performance and additional value of h-FABP according to a PTP quoted by the emergency physician. Unfortunately, our study did not show any significant gain even when restricted to low-PTP patients. In the era of other new biomarkers used in detection of AMI, such as high-sensitivity cTn or copeptin, for example, which have shown excellent results [22-24], h-FABP seems to be of little value as a biomarker in the ED.

6. Limitations

We are aware that our study presents some limitations. First, we classified our population according to an empirical clinical PTP without any standardization or accurate validation. However, this empirical classification has previously been used by other authors [14].

Second, we did not evaluate the kinetics of the biomarker because there was only 1 assay. This choice was made deliberately because we wished to test the possibility of early elimination of the diagnosis, thus avoiding serial measurements. Third, 2 different techniques were used to measure cTnI, making a comparison with other biomarkers less reliable. Thus, the ROC curve for the cTnI is a combined ROC curve of 2 different assays, making it imprecise. However, the 2 different ROC curves (for each assay) have a similar AUC and similar CI. Fourth, our study was underpowered to detect any significant change in sensitivity or NPV in our relatively small-sized subgroups. Lastly, the semiqualitative method that we used to detect positive h-FABP made the interpretation of the results somewhat artificial. The naked eye would detect bands at levels less than 3 μg/L and is more sensitive than the manufacturer's scan reader. Thus, extrapolating semiqualitative results into quantitative values is open to criticism.

7. Conclusion

In a multicenter study, h-FABP had no additional value over cTnI for the diagnosis of myocardial necrosis (STEMI and NSTEMI) in ED patients with chest pain of less than 6 hours duration. Based on previous studies, conflicting results still exist concerning the diagnostic accuracy of h-FABP. Until further positive interventional studies, the role of h-FABP remains uncertain.

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Appendice 4 : étude HsTnT + Meta analyse collaborative

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CRITICAL CARE

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High-sensitivity versus conventional troponin in the emergency department for the diagnosis of acute myocardial infarction

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Abstract

Introduction: Recently, newer assays for cardiac troponin (cTn) have been developed which are able to detect changes in concentration of the biomarker at or below the 99th percentile for a normal population. The objective of this study was to compare the diagnostic performance of a new high-sensitivity troponin T (HsTnT) assay to that of conventional cTnI for the diagnosis of acute myocardial infarction (AMI) according to pretest probability (PTP).

Methods: In consecutive patients who presented to our emergency departments with chest pain suggestive of AMI, levels of HsTnT were measured at presentation, blinded to the emergency physicians, who were asked to estimate the empirical PTP of AMI. The discharge diagnosis was adjudicated by two independent experts on the basis of all available data.

Results: A total of 317 patients were included, comprising 149 (47%) who were considered to have low PTP, 109 (34%) who were considered to have moderate PTP and 59 (19%) who were considered to have high PTP. AMI was confirmed in 45 patients (14%), 22 (9%) of whom were considered to have low to moderate PTP and 23 (39%) of whom were considered to have high PTP ($P < 0.001$). In the low to moderate PTP group, HsTnT levels ≥ 0.014 μg/L identified AMI with a higher sensitivity than cTnI (91%, 95% confidence interval (95% CI) 79 to 100, vs. 77% (95% CI 60 to 95); $P = 0.001$), but the negative predictive value was not different (99% (95% CI 98 to 100) vs. 98% (95% CI 96 to 100)). There was no difference in area under the receiver operating characteristic (ROC) curve between HsTnT and cTnI (0.93 (95% CI 0.90 to 0.98) vs. 0.94 (95% CI 0.88 to 0.97), respectively).

Conclusions: In patients with low to moderate PTP of AMI, HsTnT is slightly more useful than cTnI. Our results confirm that the use of HsTnT has a higher sensitivity than conventional cTnI.

Introduction

Early detection of acute myocardial infarction (AMI) remains a major concern, with approximately 15 million patients per year presenting to US emergency departments (EDs) with symptoms suggestive of the diagnosis [1,2]. Among such patients, a strong association between elevated cardiac troponin (cTn) levels and myocardial necrosis has been clearly demonstrated [3-5]. Conventional cTn has revolutionised the management of patients presenting with suspected acute coronary syndrome (ACS), including risk stratification of ACS, and the use of cTn measurements is recommended by current guidelines [6]. A cutoff point at the 99th percentile has been endorsed, as values above this level have repeatedly proven to be associated with adverse cardiovascular outcomes, including death [7-13]. However, the delay (4 to 6 hours, and 12 hours for peak level) in its elevation remains of concern, since it can delay AMI diagnosis and its treatment and increases the burden on EDs. Thus, cTn measurement does not reliably exclude AMI without repeated negative measurements over the course of 4 to 6 hours. These last years, newer assays have been developed, and High Sensitivity Troponin (HsTn) has been associated with higher sensitivity and

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NPV than conventional cTn. Recent studies have shown excellent diagnostic performance, even with early presentation to the ED [14], and a better diagnostic accuracy than cTn [15]. However, the latter studies did not evaluate the diagnostic accuracy of high-sensitivity troponin T (HsTnT) according to the pretest probability (PTP) of AMI. For example, ST elevation on an electrocardiogram of a patient with chest pain would be diagnosed as AMI, and then the patient would undergo cardiac catheterization without any measurement of a cardiac biomarker. Furthermore, one of the potential strengths of HsTnT might be the exclusion of AMI earlier than it would be with conventional cTn measurement as suggested by previous studies [15]. Therefore, the objectives of the current study were to confirm whether HsTnT is more sensitive than conventional cTnI to detect AMI according to the patient's PTP.

Materials and methods

Clinical setting

During the period from August 2005 to January 2007 in three urban teaching hospitals, we prospectively enrolled consecutive hospital outpatients (> 18 years of age) who presented to the ED with chest pain suggestive of ACS with the onset or peak occurring within the previous 6 hours. Patients with acute or chronic kidney failure requiring dialysis were excluded. The study was performed according to the principles of the Declaration of Helsinki and approved by the local ethics committee (Comité de Protection des Personnes Ile-de-France VI, CHU Pitié-Salpétrière Hospital, Paris, France). Because routine medical care was unchanged, waiver of informed consent was authorised. We followed most of the recommendations concerning the reporting of diagnostic studies set forth by the Standards for Reporting of Diagnostic Accuracy initiative [16].

Routine assessment

As part of the routine assessment in our institutions, all patients underwent an initial clinical evaluation that included clinical history, a physical examination, 12-lead electrocardiography (ECG), pulse oximetry, routine blood tests and chest X-rays. After these routine tests were done, and before cardiac biomarker results were available, ED physicians were asked to offer an 'empirical' clinical probability of AMI (low, medium or high PTP) based on cardiovascular risk factors, type of chest pain, physical findings and electrocardiogram abnormalities [17,18]. Conventional cardiac troponin I (cTnI) was measured at presentation and, if needed, was repeated after 3 to 9 hours as long as it was clinically indicated. Thus, according to the diagnosis of non-ST elevation MI (NSTEMI) or ST elevation MI (STEMI), the patients were admitted either to the cardiology unit for further evaluation and treatment or directly to the catheterization laboratory for primary percutaneous coronary intervention. However, the timing and treatment of patients were left to the discretion of the attending physicians according to the suspected diagnosis. ED physicians in charge were blinded to the results of HsTnT, and biologists were blinded to the emergency diagnosis suspected by physicians.

To determine the etiologic diagnosis of chest pain at presentation for each patient, two independent experts (ED physicians) who were blinded to the results of HsTnT reviewed all available medical records (including patient history, physical findings, results of laboratory and radiologic testing, ECG, echocardiography, cardiac exercise test, coronary angiography and summary chart at discharge) pertaining to the patient from the time of ED presentation to 30-day follow-up. In the event of diagnostic disagreement, cases were reviewed and adjudicated in conjunction with a third expert (also an ED physician).

AMI was diagnosed according to the joint European Society of Cardiology/American College of Cardiology/American Heart Association/World Heart Federation Task Force redefinition of MI guidelines [6]. Diagnosis of AMI required a cTnI increase above the 10% coefficient of variation (CV) value associated with at least one of the following: symptoms of ischaemia, new ST-T changes or a new Q wave on an electrocardiogram, imaging of new loss of viable myocardium or normal cTnI on admission. Unstable angina was diagnosed in patients with constant normal cTnI levels and a history or clinical symptoms consistent with ACS. Predefined further diagnostic categories included AMI (STEMI with the presence of ST-segment elevation in at least two continuous leads on ECG, new onset of left bundle branch block or NSTEMI), unstable angina, and a third group including cardiac but not coronary symptoms (for example, stable angina, myocarditis, arrhythmias and heart failure), noncardiac symptoms (for example, pulmonary embolism) and chest pain of unknown origin.

To assess the influence of renal function on cTn measurement accuracy, the creatinine level was measured in each patient and then renal function was estimated using the Modification of Diet in Renal Disease study equation [19].

Biochemical analysis

In two EDs (Cochin Hospital and La Pitié Salpêtrière Hospital, Paris, France), plasmatic cTnI concentrations were routinely measured on an Xpand HM analyzer using the Cardiac Troponin I one-step enzyme immunoassay system (Siemens Healthcare Diagnostics Inc., Newark, NJ, USA). The measurement range extended from 0.04 to 40.00 μg/L. The threshold for this method (0.14 μg/L) corresponds to the lowest substrate concentration that can be reproducibly

measured with a CV ≤ 10%. In the remaining ED (Bicêtre Hospital, Le Kremlin-Bicêtre, France), plasmatic cTnI concentrations were routinely measured on an Access analyser (Beckman Coulter, Inc., Brea, CA, USA). The measurement range of this one-step chemiluminescence immunoassay extends from 0.01 to 100.00 μg/L. The threshold (10% CV) given by the manufacturer is 0.06 μg/L.

HScTnT measurement

Heparinised samples collected upon admission and, if available, samples collected 3 to 9 hours later were analysed. Plasmatic highly sensitive cardiac TnT (HScTnT) concentrations were measured using the HScTnT one-step electrochemiluminescence immunoassay on an Elecsys 2010 analyzer (Roche Diagnostics, Meylan, France). The measuring range extended from 0.003 to 10 μg/L. The threshold for this method is 0.014 μg/L and corresponds to the 99th percentile. The CV was found to be < 10% at 0.014 μg/L. In our laboratory, CVs obtained in Roche Diagnostics quality controls containing 0.027 and 2.360 μg/L of HScTnT were < 4%. These analytical performance levels were in accordance with data provided by the manufacturer.

Statistical analysis

Continuous variables are presented as means ± SD or medians (25th to 75th percentile), and categorical variables are expressed as numbers and percentages. Continuous variables were compared by using the Mann-Whitney *U* test, and categorical variables were assessed using Pearson's χ^2 test. Correlations among continuous variables were assessed using the Spearman's rank correlation coefficient. Receiver operating characteristic (ROC) curves were constructed to assess the sensitivity and specificity, positive predictive value (PPV) and negative predictive value (NPV), positive likelihood ratio (LR^+) and negative likelihood ratio (LR^-) (all data presented with their 95% confidence intervals (95% CIs)) throughout the concentrations of cTnI and HScTnT to compare the accuracy of these markers in the diagnosis of AMI. Comparison of areas under the ROC curve was performed [20]. As this comparison is recognised as potentially insensitive, the net reclassification index (NRI) method was used as recently described [21]. For tests with binary outcomes (such as cTn for the diagnosis of AMI), NRI is defined as the gain in certainty of the first test (cTnI) minus the gain in certainty of the second test (HScTnT) or, alternatively stated, the difference of the sum of the sensitivity and specificity expressed as follows:

$$\mathrm{NRI}_{\mathrm{HScTnT\ vs.\ cTnI}} = (\text{sensitivity} + \text{specificity})_{\mathrm{HScTnT}} - (\text{sensitivity} + \text{specificity})_{\mathrm{cTnI}}.$$

NRI is the combination of four components: the proportion of individuals with events who move up or down in a category and the proportion of individuals with nonevents who move up or down in a category. Table 1 is a contingency table comparing diagnostic classifications according to cTnI and HsTnT, with shifts between the two classifications, to represent the possible benefit of HScTnT in terms of the number of patients correctly reclassified. As stated in the Routine assessment subsection above, we separated the study population into two groups: one included the patients assessed as having low or moderate PTP of AMI and the other assessed as having high PTP of AMI.

All hypothesis testing was two-tailed, and $P < 0.05$ was considered statistically significant. Statistical analysis was performed using StatView for Windows version 5.0 software (SAS Institute, Cary, NC, USA) and MedCalc software for ROC analysis (MedCalc Software, Mariarkerke, Belgium). Graphs were built with GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA).

Results

After 18 months, 317 consecutive patients were enrolled in the study. The baseline characteristics of the patients are shown in Table 2. The mean age of the patients was 57 ± 17 years (range, 40 to 90 years), and 205 (65%) were men. There were significant proportions of older adult patients (31% patients were age 65 years or older, *n* = 98) and patients with a history of cardiovascular events (26%, *n* = 83). Chest pain was considered typical of ACS in 43% (*n* = 136) of the patients. In our study

Table 1 Contingency data according to pretest probability[a]

	All patients		
Patient characteristics	**AMI**	**No AMI**	**Total**
Positive cTnI	32	9	41
Negative cTnI	13	263	276
Total	45	272	317
Positive HsTnT	42	48	90
Negative HsTnT	3	224	227
Total	45	272	317
	Low to moderate PTP		
	AMI	**No AMI**	**Total**
Positive cTnI	17	7	24
Negative cTnI	5	229	234
Total	22	236	258
Positive HsTnT	20	36	56
Negative HsTnT	2	200	202
Total	22	236	258

[a]Net reclassification improvement (NRI) from the use of highly sensitive troponin T (HsTnT) was 7.9% (95% CI = 0.9 to 14.9; $P = 0.034$). Comparison of the model including HsTnT with cTnI was significant for low PTP patients (NRI = 10.3%, 95% CI = 1.9 to 18.7; $P = 0.027$), but NRI was not significantly different in moderate PTP patients (NRI = 11.6%, 95% CI = -0.5 to 23.7; $P = 0.084$) or in high PTP patients (NRI = -14.4%, 95% CI = -32.6 to -3.6; $P = 0.181$).

Table 2 Baseline characteristics of the population according to the pretest probability[a]

Population characteristics	All patients	Low or moderate PTP	High PTP	*P* value*
Number of patients	317	258	59	
Age, years	57 ± 17	56 ± 17	60 ± 17	0.168
Men	205 (65)	166 (64)	39 (66)	0.88
Systolic BP, mmHg	141 ± 28	141 ± 27	144 ± 30	0.396
Diastolic BP, mmHg	80 ± 16	80 ± 16	82 ± 16	0.428
Heart rate, beats/minute	85 ± 45	84 ± 23	80 ± 19	0.177
Pulse oxymetry, %	97 ± 3	97 ± 3	97 ± 2	0.651
TIMI risk score	1 (0 to 3)	1 (0 to 2)	2 (1 to 4)	< 0.001
Family history of CAD	100 (32)	77 (30)	23 (59)	0.161
Personal history of CAD	83 (26)	56 (22)	27 (46)	0.0003
Dyslipidemia	113 (36)	86 (33)	27 (46)	0.069
Smoking	128 (40)	99 (38)	29 (49)	0.145
Diabetes	44 (14)	31 (12)	13 (22)	0.059
Hypertension	116 (37)	89 (34)	27 (46)	0.134
History of heart failure	21 (7)	14 (5)	7 (12)	0.083
Typical thoracic pain	136 (43)	105 (41)	31 (53)	0.11
Positive cTnI at admission	41 (13)	24 (9)	17 (29)	< 0.001**
eGFR, mL/minute/1.73 m^2	77 (62 to 94)	77 (64 to 94)	76 (56 to 91)	0.187
Treatment within first 24 hours after admission				
Aspirin	119 (38)	79 (31)	40 (68)	<0.001
Clopidogrel	54 (17)	29 (11)	25 (42)	< 0.001
LMWH	68 (21)	41 (16)	27 (46)	< 0.001
Anti GPIIb/IIIa	3 (1)	1 (0)	2 (3)	0.09
Coronarography	83 (26)	51 (20)	32 (54)	< 0.001
Outcomes				
Hospital admission	192 (61)	140 (54)	52 (88)	< 0.001
Admission to CCU	134 (42)	88 (34)	46 (78)	< 0.001
Final diagnosis				
AMI	45 (14)	22 (9)	23 (39)	< 0.001
STEMI	13 (4)	0 (0)	13 (22)	< 0.001
NSTEMI	32 (10)	22 (9)	10 (17)	< 0.001
Unstable angina	11 (3)	4 (2)	7 (12)	< 0.001
Other diagnosis	261 (82)	232 (90)	29 (49)	< 0.001***

[a]AMI, acute myocardial infarction; BP, blood pressure; CAD, coronary artery disease; cTnI, conventional troponin I; eGFR, estimated glomerular filtration rate; LMWH, low-molecular-weight heparin; anti-GPIIb/IIIa, Anti-glycoprotein IIb-IIIa; CCU, cardiologic care unit; NSTEMI, non-ST elevation myocardial infarction; PTP, pretest probability; STEMI, ST elevated myocardial infarction. TIMI, Thrombolysis in Myocardial Infarction. Results are expressed as means ± standard deviations, medians (25th to 75th percentile) or *n* (%); *statistical comparisons are between low to moderate PTP and high PTP groups unless otherwise indicated; **$P > 0.14$ μg/L in Pitie-Salpetriere and Cochin, $P > 0.06$ μg/L in Bicêtre; ***Statistical comparison including stable angina ($n = 63$), pulmonary embolism ($n = 16$), myopericarditis ($n = 43$), heart failure ($n = 5$) and others.

population, 149 patients (47%) were assessed as having a low PTP of AMI, 109 patients (34%) were assessed as moderate and 59 patients (19%) were assessed as high. AMI was confirmed in 45 patients (14%), 13 of whom had sustained STEMI, and all of these 13 patients were in the high PTP group; 32 of the patients had sustained NSTEMI. Table 2 shows that patients in the two groups (high PTP and low or moderate PTP) had significantly different characteristics. There was a higher rate of a personal history of AMI in the high PTP group and a higher final diagnosis of AMI (39% vs. 9%) in the high PTP group ($P < 0.001$). At 30 days after admission, there were three deaths (two in the AMI group and one in the other cause group) and four relapses of ACS (all in the AMI group).

HsTnT diagnostic performances

The area under the ROC curve (AUC) for the diagnosis of AMI was 0.940 (95% Confidence Intervall 0.901 to 0.980) ($P < 0.001$) for initial cTnI compared to 0.926

(0.881 to 0.971) ($P < 0.001$) for HsTnT. However, there was no significant difference between AUCs (Figure 1). ROC analysis indicated an optimal threshold of HsTnT for the diagnosis of AMI at 0.014 μg/L, with a high sensitivity of 89% (78 to 98) and a high specificity of 82% (78 to 87). The overall diagnostic accuracy of HsTnT was not significantly different compared to that of cTnI, regardless of PTP. Similar results (data not shown) were observed when we considered only NSTEMI patients (that is, after exclusion of the 13 STEMI patients). For the diagnosis of AMI, the sensitivities of HsTnT were higher and the specificities were lower than those of cTnI, regardless of PTP (Table 3). When we assessed the low and moderate PTP populations, the sensitivity of HsTnT was higher (91% (79 to 100) vs. 77% (60 to 95)) but NPV was not (99% (96 to 100) vs. 98% (95 to 99) for cTnI).

Net reclassification improvement

Table 3 shows patient classification on the basis of using cTnI or HsTnT to diagnose AMI and highlights the shifts between the two classifications.

Influence of renal function on cTn performances

Patients were classified into tertiles: tertile 1 (estimated glomerular filtration rate (eGFR) < 67.2 ml^{-1} $minute^{-1}$ 1.73 m^{-2}), tertile 2 (eGFR from 67.2 to 86.8 ml^{-1} $minute^{-1}$ 1.73 m^{-2}) and tertile 3 (eGFR ≥ 86.9 ml^{-1} $minute^{-1}$ 1.73 m^{-2}). Cardiac TnI levels were not significantly different across tertiles. However, HsTnT increased significantly across tertiles ($P < 0.001$): the lower the eGFR, the higher the HsTnT value. However, in each eGFR tertile, cTnI and HsTnT levels remained significantly

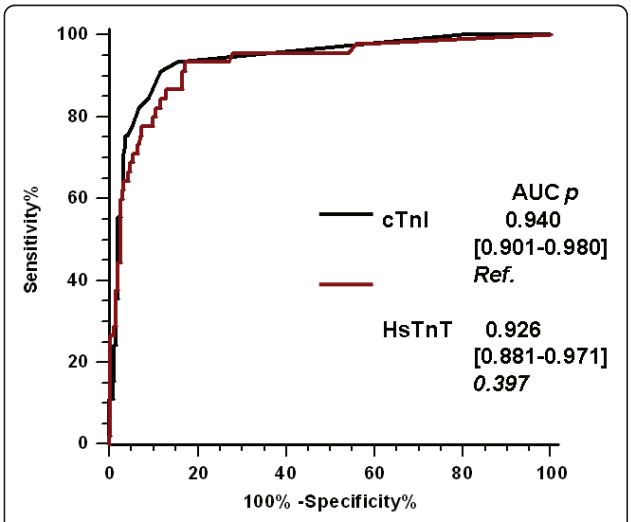

Figure 1 ROC curves for the diagnosis of AMI. Values were log-transformed before analysis. AUC: area under the curve; cTnI: conventional troponin I; HSTnT: highly sensitive troponin T.

Table 3 Diagnostic accuracy of HScTnT compared to that of cTnI for the diagnosis of AMI according to pretest probability[a]

Patient characteristics	Sensitivity	Specificity	PPV	NPV	Acc	LR^+	LR^-
All patients ($N = 317$)							
Positive cTnI	71 (55-84)	97 (94 to 98)	78 (62 to 89)	95 (92 to 97)	93 (90 to 96)	21.5 (20.1 to 22.9)	0.32 (0.23 to 0.36)
Positive HScTnT	93 (89 to 100)*	82 (77 to 87)*	47 (36 to 58)*	99 (96 to 100)	84 (79 to 88)*	5.3 (4.8 to 5.8)	0.08 (0.04 to 0.12)
Low to moderate PTP group ($n = 258$)							
Positive cTnI	77 (54 to 92)	97 (94 to 99)	71 (49 to 87)	98 (95 to 99)	95 (92 to 97)	26.1 (24.0 to 28.1)	0.23 (0.17 to 0.30)
Positive HScTnT	91 (69 to 98)	85 (79 to 89)*	36 (24 to 49)**	99 (96 to 100)	85 (80 to 89)*	6.0 (5.3 to 6.6)	0.11 (0.06 to 0.15)
High PTP group ($n = 59$)							
Positive cTnI	65 (43 to 83)	94 (79 to 99)	88 (62 to 98)	81 (65 to 91)*	83 (71 to 91)**	11.7 (10.1 to 13.4)	0.37 (0.18 to 0.55)
Positive HScTnT	96 (76 to 100)***	67 (49 to 81)***	65 (47 to 81)	96 (78 to 100)	78 (65 to 87)	2.9 (2.3 to 3.4)	0.07 (0 to 0.17)

[a]HScTnT, highly sensitive cardiac troponin T; PPV: positive predictive value; NPV: negative predictive value; Acc: diagnostic accuracy; LR: likelihood ratio. Values are expressed as percentages (except for LR) and their 95% confidence intervall. Positive cTnI > 0.14 μg/L in Pitie-Salpetriere and Ccochin, > 0.06 μg/L in Bicetre; positive HScTnT >0.014 μg/mL. *$P < 0.05$ versus positive cTnI in all patients; **$P < 0.05$ versus cTnI in low to moderate PTP group; ***$P < 0.05$ versus cTnI in high PTP group.

different between AMI and no AMI ($P < 0.001$ for both) (Figure 2). We found no significant differences in the AUCs of cTnI and HsTnT regarding eGFR tertiles, and the optimal threshold value of cTnI did not change across tertiles. Conversely, the optimal threshold value of HsTnT increased only in tertile 1 (0.036 μg/L compared to 0.014 μg/L).

Discussion

During the past two decades, cTn has been adopted as the preferred biomarker for the diagnosis of acute MI, a position reaffirmed in recent consensus guidelines [14,22]. However, until recently, cTn methods were unable to deliver the requisite analytic performance at the 99th percentile, an extremely low cutoff point within the range of analytic 'noise' in most conventional assays. The present prospective multicenter study of unselected patients who presented to the ED with chest pain of < 6 hours' duration produced major different findings about the new HsTnT assay.

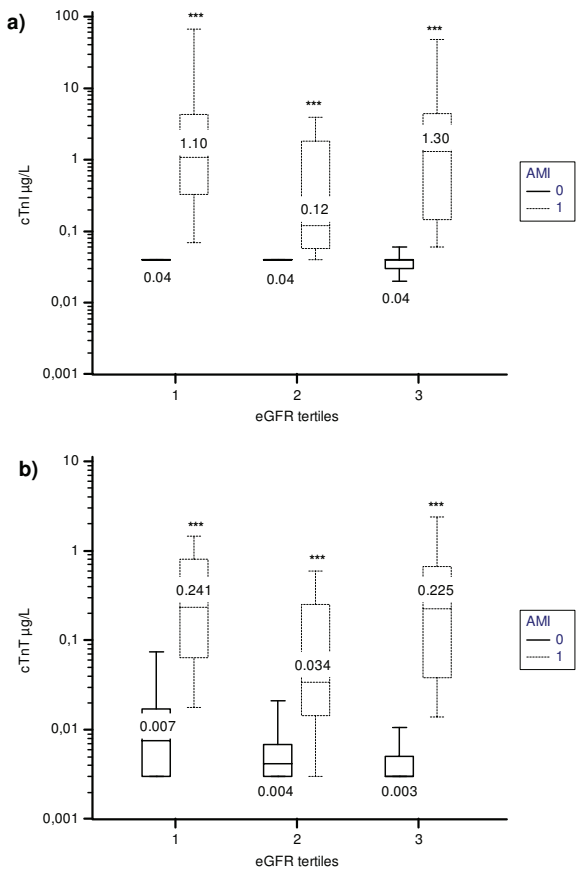

Figure 2 Boxplots for cTnI (A) and HSTnT (B) values as a function of AMI and according to eGFR tertiles. ***$P < 0.001$ versus AMI patients in the same eGFR tertile. Tertile 1 (eGFR < 67.2 mL^{-1} $minute^{-1}$ 1.73 m^{-2}), tertile 2 (eGFR from 67.2 to 86.8 mL^{-1} $minute^{-1}$ 1.73 m^{-2}) and tertile 3 (eGFR ≥ 86.9 mL^{-1} $minute^{-1}$ 1.73 m^{-2}). Medians are indicated for each box.

First, the sensitivity of the HsTnT assay remains high at all PTP levels. The excellent sensitivity of 93% was comparable to that found in a previous study (84% to 90% [22]) and significantly higher than conventional cTn (69% in our study and 72% previously described [14]). However, despite its good sensitivity of 91% in the low and moderate PTP groups, the use of HsTnT assays would not allow physicians to rule out AMI in these patients with a unique measurement of HsTnT, as the NPV is not quite perfect, that is, a unique value < 0.014 μg/L cannot avoid a second blood test several hours later to control HsTnT level. It should be noted that in the high PTP group, HsTnT showed excellent diagnostic accuracy, with 93% sensitivity (compared to 80% for cTnI) and 96% NPV (compared to 93% for cTnI). Recently, Januzzi *et al.* [15] showed that HsTnT was able to detect ACS more sensitively than a corresponding conventional cTnT method in a population of low to moderate PTP patients with chest pain.

Second, we confirmed the value of 0.014 μg/L as an optimal threshold [14,22]. We confirmed the high diagnostic accuracy of HsTnT; the AUC of HsTnT was 0.93, similar to that found by investigators in previous studies. Thus, Keller *et al.* [22] and Reichlin *et al.* [14] found AUCs that ranged from 0.94 to 0.96. However, and conversely to other reports, our findings do not show a better AUC for HsTnT than for conventional cTnI measurements. Several reasons could explain this discrepancy.

First, we used cTnI (from Siemens and Beckman Coulter) instead of cTnT as the comparator, thus with a different assay than was previously used, and our comparator cTnI could have slightly better analytical qualities than the one called the 'standard assay' that was used in the Reichlin *et al.* study [14]. Second, in our study, the AUC for cTnI, or 'conventional troponin', that is, the comparator, was 0.94 (95% CI, 0.90 to 0.98), which in fact is included in the 95% CIs of the AUCs of other comparators previously used. For example, Christ *et al.* [23] found an AUC of the standard fourth-generation cTnT assay, that is, its comparator, of 0.89 (95% CI, 0.81 to 0.98). Unfortunately, Keller *et al.* [22] did not detail the 95% CIs of their AUCs for cTn, and Reichlin *et al.* [14] used an old standard assay which in fact underestimated the diagnostic performance of the cTn assay. Other reasons could explain this discrepancy in the AUC of ROC curves for cTnI. Our inclusion criteria differ from those of Reichlin *et al.* [14], Keller *et al.* [22] and others who included patients with chest pain of less than 12 hours' duration with high rates of AMI and unstable angina. Our population markedly differs from those in previous studies. Thus, other conventional cTnT assays (also called third-generation cTnTs, from

Roche Diagnostics) that could be used in studies as comparators for HSTnT have been reported to have excellent AUCs. Collinson *et al.* [24] found that at 6 hours postpain, the AUC of cTnT was 0.989 (95% CI, 0.966 to 1.0). However, although the comparison of AUCs remains the most popular metric by which to capture discrimination, it appears that for models containing clinical risk and possessing reasonably good discrimination, very important associations between the biomarker and the end point are required to provide significantly different AUCs. In other words, comparisons of AUCs might be considered powerless in identifying biomarkers of interest in such situations [20]. To address this problem, new ways of evaluating the usefulness of biomarkers have been described, but they are used very rarely in studies evaluating diagnostic tests or biomarkers [14,22]. In the present study, reclassification, for example, NRI, demonstrated that the use of HsTnT with a clinical assessment (including ECG findings) only slightly improved the discriminative power and performance in predicting AMI [14,22,25]. As described in previous studies, we have demonstrated a worsening of specificity and lower PPV of HsTnT measurement compared to those of conventional cTn; that is, we observed an increase in false-positive findings. Last, the present study is the first to investigate the impact of kidney function on HsTnT levels. We found no significant difference in the AUCs of HsTnT regarding eGFR tertiles. Only in tertile 1 was the optimal threshold value of HsTnT increased (0.036 μg/ml compared to 0.014 μg/L).

Conventional cTn is widely used and is recommended for the management of patients presenting with suspected ACS [6]. However, the delay in detecting its elevation prevents early, safe discharge from the ED without repeated negative measurements during the course of 4 to 6 hours. Recent studies have shown excellent diagnostic performance of HsTnT measurement, even with early presentation to the ED [14], and better diagnostic accuracy than cTn [15]. Despite its higher sensitivity, we did not find that HsTnT had better NPV, diagnostic accuracy or AUC, conversely to the findings of previous studies [15]. Furthermore, as expected, specificity and PPV were lower. The clinical setting, time of inclusion, rate of AMI in our patient population and our focus on low or moderate PTP of AMI could explain this discrepancy.

The emergency medicine field would greatly benefit from a new biomarker that eases and hastens the triage of noncardiac chest pain patients. The main incremental value that could have provided a new highly sensitive assay for Tn would have allowed emergency physicians to rule out AMI and discharge patients with a normal Tn value. This study suggests that even when considering only low to moderate PTP patients, the better sensitivity of HsTnT cannot translate into a real clinical improvement. A NPV of 99% can be interpreted as excellent, but this slight gain from that of cTnI is not sufficient to change the conventional method of chest pain investigation in our ED, even in low to moderate PTP patients. This subgroup is the one of most interest in our study, as high PTP patients (and even more so for STEMI patients) are not to be promptly discharged and will more easily undergo further investigations and care.

To rapidly and reliably rule out AMI, the answer may be assessment of a combination of different biomarkers, as suggested by Reichlin *et al.* [26] in their study, where they found that with a copeptin level < 14 pmol/L and a TnT level < 0.01 μg/L, AMI was excluded with 99.7% NPV in an unselected population of chest pain patients.

Limitations

The main limitation of our study is the small sample of patients, especially patients with AMI. We cannot exclude the possibility that better results might have been found with a larger sample. Our sample is comparable to those used in previous studies, however, and most of all, we believe that the imperfect NPV that we describe herein is the major result of our study, which could not have been corrected by including more patients.

Our study has some other limitations. First, we performed only a single measurement of HsTnT. We did not evaluate its kinetics, which would have been interesting, especially in the 'grey zone' (between 0.014 μg/L and 0.050 μg/L). A second value could have provided more data, as previously described in the Giannitsis *et al.* study [27], which reported that a doubling in the HsTnT concentration within 3 hours of chest pain (with first negative HsTnT and no electrocardiogram abnormality) was associated with a 100% PPV of a diagnosis of NSTEMI.

Second, we used empirical PTP and not a standardised, validated one [17,18]. However, outcomes in the low and moderate PTP population (only nine with confirmed NSTEMI), and differences in clinical characteristics at admission suggested that even though empirical, this evaluation by the clinician was accurate. Furthermore, one of the strengths of our study was that it evaluated differences in diagnostic performance for the HsTnT regarding PTP as demonstrated for D-dimers and empirical suspicion of pulmonary embolism [28]. Another limitation of our study is that different conventional Tn assays have been used at the two study sites with different threshold values and CVs. These assays were used because they were both local and well-understood methods at the time of the study.

Third, we used two different assays for the comparator (that is, conventional TnI): a Siemens cTnI assay in two centres (CCH and PSL) and a Beckman Coulter assay in

the third centre (BCT). The ROC curve for the cTnI is, then, a combined ROC curve of two different assays, making it imprecise. However, the two different ROC curves (for each assay) have similar AUCs.

Last, this study was underpowered to find any significant change in the detection of AMI in the low to moderate PTP patients. However, as the NPV is not perfect in our patient population, we expect that this would remain the case with a larger sample.

Conclusions

We have confirmed that HsTnT is accurate for diagnosis of AMI, with a sensitivity slightly higher than that of conventional cTnI, regardless of PTP of AMI in patients with chest pain presenting to an ED. However, we did not show a better NPV. Intervention studies are clearly warranted to support the use of HsTnT to help ED physicians achieve clinical improvement in treating patients with chest pain and providing them with an early, safe discharge from the hospital.

Key messages

- Fast and reliable detection of ACS remains a great concern in the ED.
- Novel assays for troponin have been developed and tested recently.
- HsTnT is more sensitive than cTn.
- In this study, the weak gains realised by measuring HsTnT rather than cTn in terms of NPV is not sufficient to change daily clinical practice.

Abbreviations

ACS: acute coronary syndrome; AMI: acute myocardial infarction; AUC: area under the curve; cTn: conventional troponin; CV: coefficient of variation; ED: emergency department; HsTn: high-sensitivity troponin; LR: likelihood ratio; NPV: negative predictive value; NRI: net reclassification improvement; NSTEMI: non-ST elevation myocardial infarction; PPV: positive predictive value; PTP: pretest probability; ROC: receiver operating characteristic; SD: standard deviation; STEMI: ST elevation myocardial infarction.

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Authors' contributions

CCG, BR and PR designed the study. PB, YEC, JCA, BD, FL and CC helped in collecting the data. CC and YF carried out the statistical analyses and the biochemical assays. YF, CCG, BR and PR wrote the paper. All authors read and approved the final manuscript.

Competing interests

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Comparison of conventional and high-sensitivity troponin in patients with chest pain: A collaborative meta-analysis

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Background Multiple studies have evaluated the diagnostic and prognostic performance of conventional troponin (cTn) and high-sensitivity troponin (hs-cTn). We performed a collaborative meta-analysis comparing cTn and hs-cTn for diagnosis of acute myocardial infarction (AMI) and assessment of prognosis in patients with chest pain.

Methods MEDLINE/PubMed, Cochrane CENTRAL, and EMBASE were searched for studies assessing both cTn and hs-cTn in patients with chest pain. Study authors were contacted and many provided previously unpublished data.

Results From 17 included studies, there were 8,644 patients. Compared with baseline cTn, baseline hs-cTn had significantly greater sensitivity (0.884 vs 0.749, $P < .001$) and negative predictive value (NPV; 0.964 vs 0.935, $P < .001$), whereas specificity (0.816 vs 0.938, $P < .001$) and positive predictive value (0.558 vs 0.759, $P < .001$) were significantly reduced. Based on summary receiver operating characteristic curves, test performance for the diagnosis of AMI was not significantly different between baseline cTn and hs-cTn (0.90 [95% CI 0.85-0.95] vs 0.92 [95% CI 0.90-0.94]). In a subanalysis of 6 studies that alternatively defined AMI based on hs-cTn, cTn had lower sensitivity (0.666, $P < .001$) and NPV (0.906, $P < .001$). Elevation of baseline hs-cTn, but negative baseline cTn, was associated with increased risk of death or nonfatal myocardial infarction during follow-up ($P < .001$) compared with both negative.

Conclusion High-sensitivity troponin has significantly greater early sensitivity and NPV for the diagnosis of AMI at the cost of specificity and positive predictive value, which may enable early rule in/out of AMI in patients with chest pain. Baseline hs-cTn elevation in the setting of negative cTn is also associated with increased nonfatal myocardial infarction or death during follow-up. (Am Heart J 2014;169:6-16.e6.)

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More than 7 million patients present annually to the emergency department (ED) with chest pain,[1] and >1 million patients are hospitalized each year in the United States with acute myocardial infarction (AMI).[2] The ability to rapidly exclude AMI through high-sensitivity troponin (hs-cTn) in combination with clinical evaluation may reduce ED length of stay, reduce financial cost, and improve outcomes in these challenging patients. Evidence suggests that even minimal elevations of conventional troponin (cTn) are associated with worse clinical outcome and that these patients may benefit from initiation of appropriate medical intervention.[3,4] Furthermore, use of a very low cut-point for hs-cTn has been suggested as a tool to rule out AMI due to the resulting high negative predictive value (NPV).[5] However, the introduction of hs-cTn may significantly decrease specificity and can prompt a costly cardiovascular workup in patients in which cTn is elevated due to nonischemic causes for cTn release. Although multiple studies have compared the diagnostic and prognostic test characteristics of cTn and hs-cTn, the results of these data are mixed. Therefore, we performed a diagnostic and prognostic collaborative meta-analysis to assess cTn values and hs-cTn values in patients with chest pain.

Methods

Data sources and searches

Two independent reviewers (M.J.L. and N.C.B.) systematically searched (November 2013) Cochrane CENTRAL, EMBASE, and MEDLINE/PubMed for studies that assessed both cTn and hs-cTn in patients with nontraumatic chest pain. Search criteria included "high sensitivity troponin" AND ("chest pain" OR "acute coronary syndromes" [ACS] OR "myocardial infarction"). We limited our search to studies published in peer-reviewed journals; trials presented in abstract-only form were excluded. Our meta-analysis was performed in accordance with the Meta-Analysis Of Observational Studies in Epidemiology guidelines.[6] After obtaining full reports, eligibility was assessed from the full-text articles with divergences resolved after consensus. No extramural funding was used to support this work. The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the manuscript, and its final contents.

Study selection

Prespecified inclusion and exclusion criteria were established at study onset. We included any study that (*a*) assessed patients with nontraumatic chest pain and (*b*) measured both cTn and hs-cTn levels. We excluded any study that (*a*) limited patients to only those with myocardial infarction (MI) or a specific subgroup of patients, (*b*) excluded patients with a baseline positive troponin, and (*c*) used a case-control format. We included studies regardless of whether patients with ST-segment elevation MI (STEMI) were included or excluded, whether the criterion standard diagnosis was made centrally or locally, and regardless of the cTn criteria used for diagnosis of AMI.

Data extraction and quality assessment

Data were abstracted by the same 2 investigators (M.J.L. and N.C.B.). An attempt was made to contact the corresponding authors of included studies to obtain complete data. Study quality was appraised in accordance with QUality Assessment of Diagnostic Accuracy Studies (QUADAS)-2.[7] We accepted the authors' definitions of conventional and hs-cTn.

Data synthesis and analysis

Dichotomous variables are reported as proportions (percentages), whereas continuous variables are reported as mean (SD) or median. Sensitivity, specificity, positive predictive values (PPV), NPVs, positive and negative likelihood ratios (LRs), and diagnostic odds ratios (ORs) were computed. Pooling was performed using random-effects methods. Measures of test performance are reported as point estimates (with 95% CIs). These were calculated for the baseline cTn at presentation, baseline hs-cTn at presentation, cTn at the second serial sampling (second cTn), and hs-cTn at the second serial sampling (second hs-cTn). Adjudication of AMI was typically defined by cTn. Given that authors used their own cut-points and delta changes in troponin with different times for sampling, we were unable to assess for value of serial sampling in this meta-analysis.

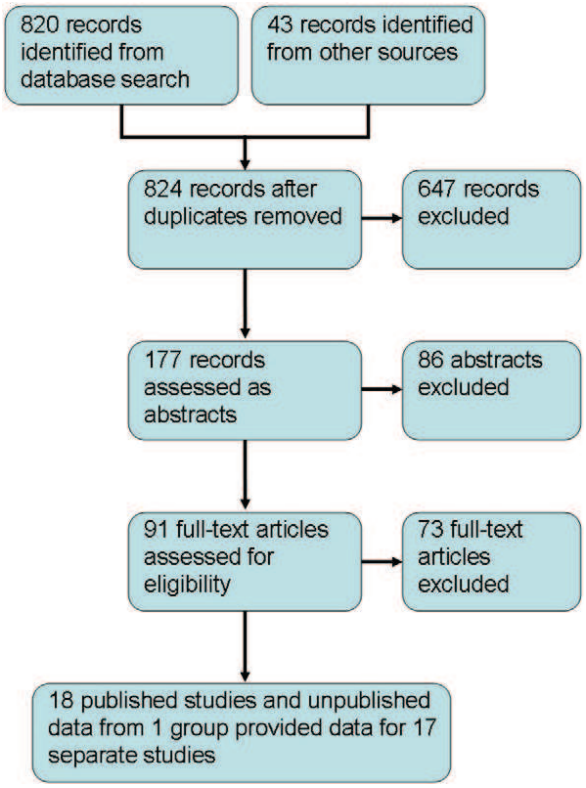
We generated weighted symmetric summary receiver operating characteristic (SROC) plots using the Moses-Shapiro-Littenberg method.[8] Area under the ROC curves of individual studies were pooled using a random effect generic-inverse variance method. Sources of clinical and statistical heterogeneity were explored by means of subgroup analyses and meta-regression with unrestricted maximum-likelihood meta-regression (inverse variance-weighted regression) on diagnostic ORs.

Binary outcomes from individual studies were combined with random-effect models, leading to computations of ORs with 95% CIs. Between-study statistical heterogeneity was assessed using the Cochran Q χ^2 test. I^2 was calculated as a measure of statistical heterogeneity; I^2 values of 25%, 50%, and 75% represented mild, moderate, and severe inconsistency, respectively. Small study or publication bias was explored with funnel plots and Peters test.[9] Statistical analysis was performed using Review Manager (RevMan) 5 version 5.1.7 freeware package (The Nordic Cochrane Centre, The Cochrane Collaboration, 2008, Copenhagen, Denmark), Meta-DiSc software,[10] and NCSS 2007 (Kaysville, UT), with statistical significance for hypothesis testing set at the .05 two-tailed level and for heterogeneity testing at the .10 two-tailed level.

Results

Of the 824 citations we identified, we assessed 177 abstracts from which we performed detailed review of 91

Figure 1



Flow diagram of study selection.

full-text manuscripts. Articles were excluded if the study was limited to only patients with stable coronary artery disease or only patients with ACS, patient duplication, exclusion of patients with baseline positive troponins, use of a case-control design, lack of or inadequate cTn data, and lack of adjudication data for AMI (excluded studies are listed in the supplement). Authors of the APACE study (Drs Twerenbold and Mueller) provided comprehensive data not only for the patients published in Haaf et al [11] but also on an additional 416 patients to provide the most updated data from their registry. Thus, our systematic review and collaborative meta-analysis comprises data from 18 published studies[12–29] (data from 3 studies were used to compile the findings of Aldous et al.[12–14]) and updated data from the APACE study to provide comprehensive data on 17 studies. The details of our flow diagram can be found in Figure 1. Study characteristics are presented in Table I, and appraisal of diagnostic study quality can be found in online Appendix Supplementary Table I.

The 17 studies included a total of 8,644 patients (median of 332 patients [range 58-1,818]). Patient characteristics are shown in Table II. The population had a weighted mean age of 62 ± 15 years, 63% of patients were male, and there was a typical distribution of cardiovascular risk factors. Of the included patients, 20.7% were diagnosed as having AMI, with 5.2% admitted with STEMI. In studies that reported unstable angina, 13.4% of patients were diagnosed as having unstable angina. Most studies used cTn levels for the adjudication of AMI, whereas several studies used a combination of cTn and hs-cTn levels (online Appendix Supplementary Table I).

Diagnostic performance of individual studies is summarized for baseline cTn and baseline hs-cTn (online Appendix Supplementary Table II), along with the second cTn and the second hs-cTn (online Appendix Supplementary Table III). In addition to adjudicating AMI with conventional cTn, 6 studies also performed separate adjudication for AMI using the hs-cTn levels as the criterion standard to define AMI, and diagnostic performance for baseline cTn and hs-cTn is provided (online Appendix Supplementary Table IV). Finally, the area under the ROC curves for baseline cTn, baseline hs-cTn, second cTn, and second hs-cTn for diagnosis of AMI can be found in online Appendix Supplementary Table V.

Diagnostic accuracy of cTn and hs-cTn

The assays used in each study are shown in Table I. As seen in Table III, baseline hs-cTn had significantly greater sensitivity ($P < .001$) and NPV ($P < .001$), and significantly lower negative LR ($P < .01$), whereas baseline cTn had significantly greater specificity ($P < .001$), PPV ($P < .001$), and positive LR ($P < .01$). The SROC curves suggest a trend toward better diagnostic accuracy with baseline hs-cTn (Table III, Figure 2). Comparison of pooled area under the ROC curves also suggested a trend toward better performance for baseline hs-cTn compared with baseline cTn (0.91 [95% CI 0.89-0.93] vs 0.89 [95% CI 0.86-0.91], respectively; $P = .22$, $I^2 = 33\%$).

The second cTn was checked 2.6 ± 1.5 hours after the baseline cTn, and the second hs-cTn was checked 2.5 ± 1.4 hours after the baseline hs-cTn in 10 studies with 5,174 patients (online Appendix Supplementary Table III). These data demonstrated that the sensitivity remained significantly greater for the second hs-cTn compared with the second cTn ($P < .05$), whereas the second cTn had significantly greater specificity ($P < .001$), PPV ($P < .001$), and positive LR ($P < .01$) compared with the second hs-cTn (Table III). Summary receiver operating characteristic curves demonstrated no difference in diagnostic accuracy (Table III). Pooled area under the ROC curve was not significantly different between the second cTn and the second hs-cTn (0.95 [95% CI 0.93-0.97] vs 0.96 [95% CI 0.94-0.97], respectively; $P = .42$, $I^2 = 0\%$) (online Appendix Supplementary Table V). Sensitivity analyses of conventional cTn or hs-cTn with exclusion of one study at a time did not appear to significantly change the sensitivity or specificity.

Meta-regression analysis

Meta-regression demonstrated that time from onset of chest pain to presentation was significantly associated with improved test performance for baseline cTn (regression coefficient 0.61 ± SE 0.20, $P = .02$) but not

Table I. Study characteristics

Study	Year published	Patients	Centers	Inclusion criteria for chest pain	Conventional Tn assay (cut-point)	HS-Tn assay (cut-point)	Follow-Up (mo)
Aldous et al[15]	2012	939	Multi	No exclusion	Abbott Architect cTnI, 30 ng/L (10% CV, 32 ng/L)	Roche HS TnT, 14 ng/L (99th percentile)	12
Aldous et al[12-14]	2011	332	Single	No exclusion	Abbott Architect cTnI, 30 ng/L (10% CV, 32 ng/L)	Roche HS TnT, 14 ng/L (99th percentile)	24
APACE	N/A	1533	Multi	<12 h	Roche cTnT 4th gen, 35 ng/L (10% CV) but Siemens RxL TnI, 140 ng/L (10% CV) to define AMI	Roche HS TnT, 14 ng/L (99th percentile)	24
Christ et al[16]	2010	137	Single	No exclusion	Roche cTnT 4th gen, 35 ng/L (10% CV)	Roche HS TnT, 14 ng/L (99th percentile)	8
Collinson et al[17]	2013	850	Multi	No exclusion	Siemens Stratus CS cTnI, 70 ng/L (99th percentile)	Beckman AccuTnI, 40 ng/L (99th percentile)	3
Eggers et al[18]	2012	360	Multi	<8 h	Siemens Stratus CS cTnI, 70 ng/L (99th percentile)	Roche HS TnT, 14 ng/L (99th percentile)	6
Freund et al[19]	2011	317	Multi	<6 h	Siemens Xpand HM cTnI, 140 ng/L or Beckman Coulter Access cTnI, 60 ng/L (both 10% CV)	Roche HS TnT, 14 ng/L (99th percentile)	1
Hammerer-Lercher et al[20]	2013	440	Single	No exclusion	Roche Elecsys cTnT 4th gen, 10 ng/L (99th percentile)	Roche HS TnT, 14 ng/L (99th percentile)	8
Inoue et al[21]	2011	283	Multi	<24 h	Roche cTnT 4th gen, 35 ng/L (10% CV) but 100 ng/L (WHO criteria) to define AMI	Roche HS TnT, 14 ng/L (99th percentile)	No
Keller et al[22]	2009	1818	Multi	No exclusion	Roche Elecsys cTnT 4th gen, 30 ng/L (10% CV), but Siemens RxL TnI, 140 ng/L (10% CV) to define AMI	Siemens sensitive TnI Ultra, 40 ng/L (99th percentile)	1; unable to abstract
Lotze et al[23]	2011	142	Single	No exclusion	Roche cTnT 4th gen, 100 ng/L (WHO Criteria)	Roche HS TnT, 14 ng/L (99th percentile)	No
Melki et al[24]	2011	233	Single	<12 h	Roche cTnT 4th gen, 40 ng/L (10% CV, 35 ng/L)	Roche HS TnT, 14 ng/L (99th percentile)	No
Meune et al[25]	2011	58	Single	<6 h	Siemens Xpand HM cTnI, 70 ng/L (99th percentile)	Roche HS TnT, 14 ng/L (99th percentile)	No
Pracon et al[26]	2012	187	Single	<24 h	Siemens Dimension Flex TnI, 70 mg/L (99th percentile)	Abbott Architect Stat TnI, 28 ng/L (99th percentile)	No
Santalo et al[27]	2013	356	Multi	No exclusion	Roche Cobas e401 cTnT 4th gen, 10 ng/L (99th percentile)	Roche HS TnT, 14 ng/L (99th percentile)	12
Schreiber et al[28]	2012	465	Single	No exclusion	Siemens Dimension RxL TnI, 140 ng/L (10% CV)	Singulex Erenna HS-TnI, 8 ng/L (99th percentile, 10.1 ng/L)	1
Sebbane et al[29]	2013	194	Single	<12 h	Beckman Access2 cTnI, 40 ng/L (intended 99th percentile)	Roche HS TnT, 14 ng/L (99th percentile)	No

Abbott (Abbott Park, IL), Roche (Indianapolis, IN), Siemens (Tarrytown, NY), Singulex (St Louis, MO).

baseline hs-cTn (regression coefficient 0.38 ± SE 0.20, P = .10). Neither time from presentation to the second cTn nor the second hs-cTn was significantly associated with test performance. The percentage of patients with STEMI (regression coefficient −4.6 ± 1.1, P = .001), male sex (regression coefficient −8.3 ± SE 3.0, P = .02), diabetes (regression coefficient −8.0 ± SE 2.9, P = .02), and prevalence of AMI (regression coefficient −3.2 ± SE 1.2, P = .02) were significantly associated with test performance for baseline cTn but was not associated with test performance for baseline hs-cTn. Age, creatinine levels, and estimated glomerular filtration rate were not associated with test performance for baseline cTn or baseline hs-cTn. The definition of the delta, or the change by rise and/or fall of troponin, used to diagnosis AMI was also not significantly associated with test performance.

Subgroup analysis

When comparing studies that used the 10% coefficient variance (CV) cut-point[12,15,16,19,22,24,28] (see also APACE) vs 99th percentile cut-point[17,18,20,25–27,29] for cTn to define AMI, baseline cTn using 10% CV cut-point had significantly greater specificity (0.957 [0.950-0.962] vs 0.921[0.908-0.933]), PPV (0.813 [0.788-0.836] vs 0.699 [0.657-0.738]), and positive LR (15.804 [10.699-23.345] vs 8.905[5.771-13.740]) than baseline cTn using 99th percentile cut-point, with no significant differences between the groups in terms of sensitivity (0.754 [0.728-0.778] vs 0.788 [0.747-0.824]), NPV (0.940 [0.932-0.946] vs 0.949 [0.938-0.959]), negative LR (0.260 [0.218-0.311] vs 0.238 [0.192-0.294]), diagnostic OR (60.651 [36.377-101.12] vs 44.054 [26.685-72.727]), or SROC (0.889 [0.756-0.990] vs 0.919 [0.879-0.959]).

Table II. Patient characteristics of included studies

Study	Age (y)	Male	Prior CAD	Prior MI	HTN	HLD	DM	Smoking	TTP (h)	STEMI	NSTEMI	AMI	UA
Aldous et al 2012[15]	65	59.7%	51.8%	NR	60.8%	57.6%	16.5%	60.6%	6.3	0	21.8%	21.8%	NR
Aldous et al 2011[12-14]	64	60.2%	53.9%	NR	45.8%	38.0%	16.3%	17.2%	4	0	33.1%	33.1%	17.2%
APACE	63 ± 16	67.0%	36.2%	24.2%	65.9%	50.8%	19.2%	24.1%	5	3.7%	11.5%	15.3%	14.3%
Christ et al[16]	66 ± 16	63.5%	34.3%	32.8%	66.4%	35.0%	22.6%	21.9%	NR	2.9%	11.7%	14.6%	19.0%
Collinson et al[17]	54	59.6%	NR	5.8%	35.4%	23.6%	8.1%	28.5%	5.9	0	8.0%	8.0%	NR
Eggers et al[18]	66 ± 12	65.6%	42.8%	37.5%	42.8%	38.3%	18.3%	18.1%	4.5	0	35.6%	35.6%	18.9%
Freund et al[19]	57 ± 17	64.7%	31.6%	26.2%	36.6%	35.8%	13.9%	40.6%	NR	4.1%	10.1%	14.2%	3.5%
Hammerer-Lercher et al[20]	56 ± 20	52.3%	19.1%	NR	46.4%	NR	7.5%	NR	3	5.9%	3.2%	9.1%	NR
Inoue et al[21]	65 ± 12	74.0%	NR	NR	51.9%	44.2%	29.4%	35.5%	3	50.9%	6.7%	57.6%	10.2%
Keller et al[22]	61 ± 14	66.4%	35.8%	NR	73.7%	73.0%	15.7%	24.3%	NR	7.2%	15.6%	22.7%	13.2%
Lotze et al[23]	71 ± 14	76.0%	27.5%	15.5%	73.9%	16.9%	28.9%	7.7%	NR	6.3%	2.8%	9.2%	2.1%
Melki et al[24]	65	66.5%	NR	30.0%	50.2%	NR	22.7%	17.2%	5.3	0	48.9%	48.9%	12.0%
Meune et al[25]	58 ± 14	63.8%	NR	20.7%	46.7%	37.9%	22.4%	32.8%	7.5	0	22.4%	22.4%	29.3%
Pracon et al[26]	64 ± 14	63.6%	NR	17.6%	61.0%	36.4%	14.4%	13.9%	NR	23.0%	21.9%	44.9%	5.9%
Santalo et al[27]	69	67.9%	34.9%	NR	62.0%	NR	26.4%	NR	5	0	21.9%	21.9%	29.5%
Schreiber et al[28]	67	49.2%	NR	19.1%	62.2%	NR	17.4%	11.2%	NR	0	2.6%	2.6%	3.4%
Sebbane et al[29]	61 ± 17	63.4%	21.6%	14.8%	34.0%	35.1%	14.1%	36.6%	4.24	13.9%	12.4%	26.3%	16.0%
Weighted mean	62 ± 15	63.4%	37.5%	20.9%	58.1%	50.1%	16.8%	28.3%	5.1 ± 1.1	5.2%	15.5%	20.7%	13.4%

Abbreviations: *CAD*, Coronary artery disease; *HTN*, hypertension; *HLD*, hyperlipidemia; *DM*, diabetes mellitus; *TTP*, time from onset of chest pain to presentation; *NSTEMI*, non–ST elevation MI; *UA*, unstable angina.

Table III. Summary of sensitivity, specificity, PPV, NPV, positive LR, negative LR, diagnostic OR (DOR), and area under the SROC curves for the baseline and second serial conventional and hs-cTn (hs-cTn) for AMI

	Baseline cTn	Baseline hs-cTn	Second Serial cTn	Second Serial hs-cTn
Pooled sensitivity	0.749 (0.728-0.769)	0.884 (0.868-0.898)	0.895 (0.867-0.919)	0.928 (0.903-0.948)
Pooled specificity	0.938 (0.932-0.943)	0.816 (0.807-0.826)	0.952 (0.944-0.959)	0.807 (0.794-0.821)
Pooled PPV	0.759 (0.738-0.778)	0.558 (0.539-0.576)	0.758 (0.724-0.790)	0.443 (0.414-0.472)
Pooled NPV	0.935 (0.929-0.940)	0.964 (0.959-0.969)	0.982 (0.977-0.986)	0.985 (0.980-0.990)
Summary positive LR	9.913 (6.648-14.781)	4.393 (3.403-5.673)	13.163 (7.667-22.596)	4.663 (3.576-6.080)
Summary negative LR	0.262 (0.217-0.317)	0.156 (0.116-0.210)	0.137 (0.092-0.204)	0.112 (0.069-0.182)
Summary DOR	41.665 (24.732-70.191)	32.609 (20.477-51.931)	95.503 (45.727-199.46)	49.716 (25.238-97.938)
Area under the SROC curve	0.890 (0.839-0.941)	0.923 (0.899-0.947)	0.951 (0.919-0.983)	0.948 (0.912-0.984)

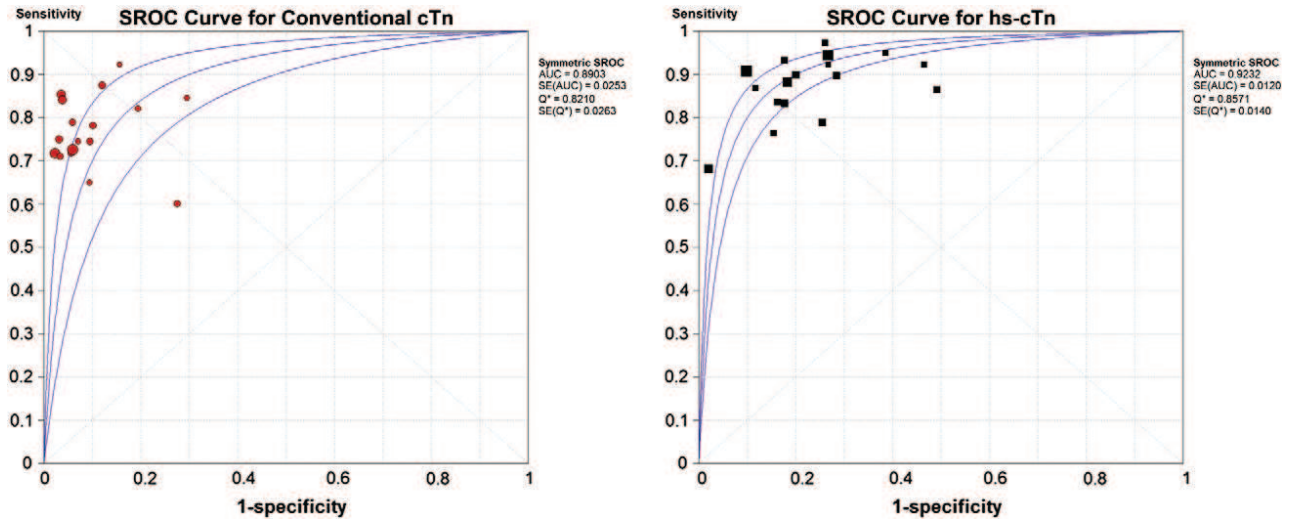
There was no significant difference in test performance for baseline cTn in studies that used a 10% CV cut-point compared with a 99th percentile cut-point to define AMI as assessed by pooled area under the ROC curves (0.90 [0.86-0.93] vs 0.91 [0.88-0.93], $P = .61$, $I^2 = 0\%$).

When comparing the diagnostic performance of baseline cTnT[16,20,23,24,27] (see also APACE) and cTnI[12,15,17–19,25,26,28,29] to define AMI, baseline cTnT had significantly lower specificity (0.931 [0.920-0.941] vs 0.950[0.941-0.957]) and PPV (0.701 [0.661-0.740] vs 0.790 [0.759-0.820]) compared with baseline cTnI. There were no differences between baseline cTnT and baseline cTnI in sensitivity (0.758 [0.717-0.795] vs 0.790 [0.759-0.820]), NPV (0.947 [0.938-0.956] vs 0.950 [0.941-0.957]), positive LR (8.822 [3.996-19.478] vs 12.532 [7.848-20.010]), negative LR (0.263 [0.20-0.314] vs 0.235 [0.189-0.292]), diagnostic OR (42.289 [21.696-82.428] vs 57.519 [32.471-101.89]), or SROC (0.904 [0.860-0.948] vs 0.917 [0.863-0.971]). There was no significant difference in test performance for baseline cTnT and baseline cTnI as assessed by pooled area under the ROC curves (0.89 [0.86-0.93] vs 0.91 [0.89-0.93], $P = .30$, $I^2 = 7.1\%$).

AMI definition based on hs-cTn

When limiting studies to those that provided a separate adjudication using hs-cTn to define AMI,[12,14,16,17,24,27] (see also APACE), the mean prevalence of AMI increased from 23% ± 15% when AMI was defined by cTn to 29.6% ± 16.5% when AMI was defined by hs-cTn. When AMI was defined by hs-cTn, the baseline hs-cTn had significantly greater test performance based on pooled area under the ROC curves compared with baseline cTn (0.91 [95% CI 0.88-0.94] vs 0.80 [95% CI 0.74-0.87], respectively; $P = .004$). Baseline cTn had a significant reduction in sensitivity (0.666 vs 0.749, $P < .001$) and NPV (0.906 vs 0.935, $P < .001$) when AMI was defined by hs-cTn compared with when AMI was defined by cTn. Baseline hs-cTn also had a significant reduction in sensitivity (0.857 vs 0.884, $P < .05$) and NPV (0.953 vs 0.964,

Figure 2



Summary ROC curves for the baseline conventional cTn (left) and baseline hs-cTn (right), which plots sensitivity and 1 – specificity for each study, enabling comparison of the 2 assays. Studies were weighted by least-squares method using the inverse variance. Studies are plotted for conventional cTn with red circles and plotted for hs-cTn with black squares. Symmetric SROC curves are present with a 95% CI, and area under the SROC curve is provided along with SEs to the right in each figure.

Table IV. Summary of sensitivity, specificity, PPV, NPV, positive LR, negative LR, diagnostic OR (DOR), and area under the summary SROC curves for cTn and hs-cTn when AMI is based on using the cut-point for hs-cTn

	Baseline cTn	Baseline hs-cTn
Pooled sensitivity	0.666 (0.631-0.699)	0.857 (0.830-0.881)
Pooled specificity	0.941 (0.931-0.950)	0.854 (0.840-0.868)
Pooled PPV	0.768 (0.734-0.799)	0.632 (0.602-0.661)
Pooled NPV	0.906 (0.894-0.916)	0.953 (0.944-0.962)
Summary positive LR	8.797 (3.892-19.888)	7.482 (4.114-13.608)
Summary negative LR	0.314 (0.205-0.479)	0.145 (0.070-0.304)
Summary DOR	30.004 (14.080-63.937)	57.034 (24.958-130.33)
Area under the SROC curve	0.904 (0.817-0.991)	0.945 (0.907-0.983)

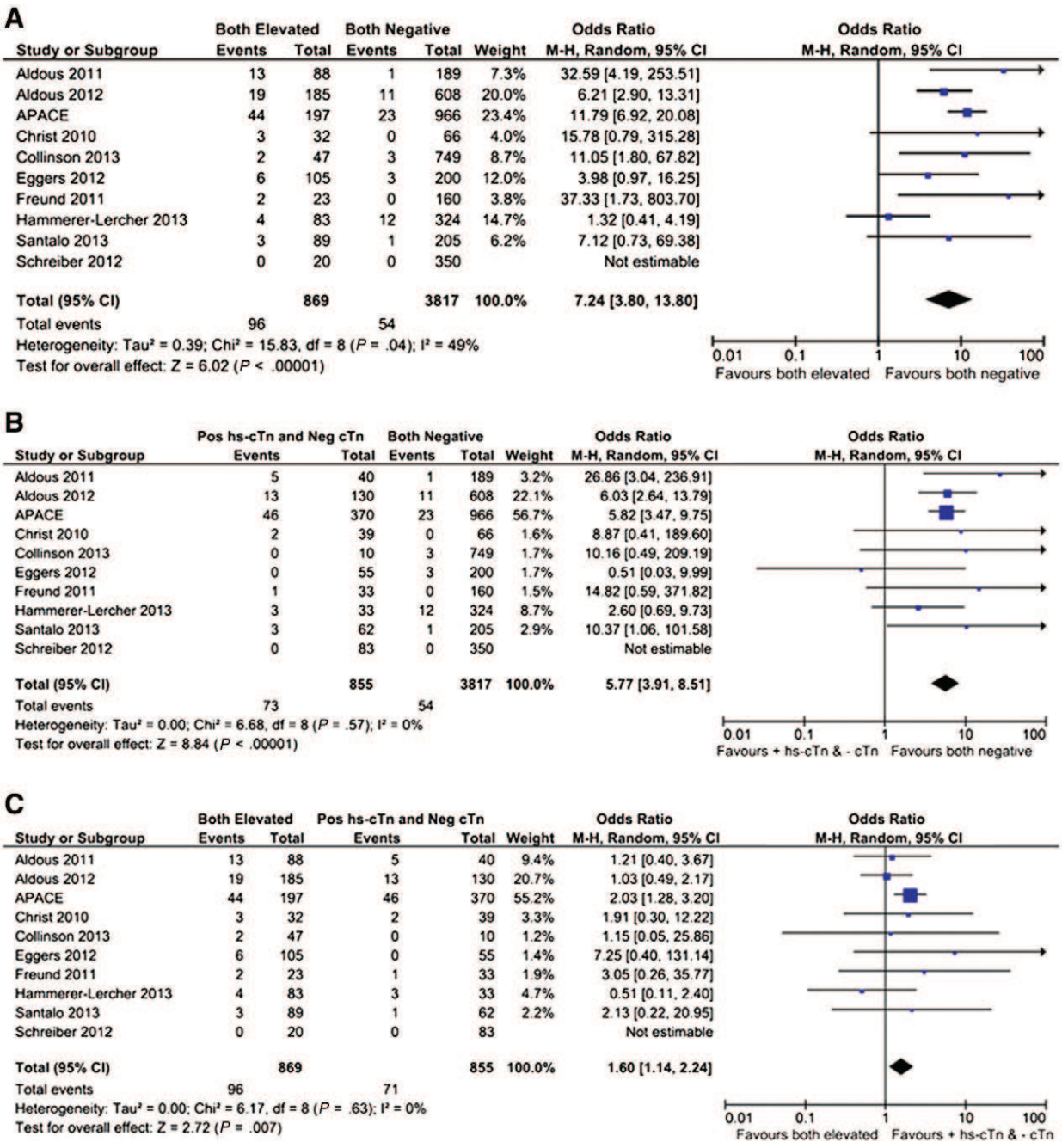
$P < .05$) with an increase in specificity (0.854 vs 0.816, $P < .001$) and PPV (0.632 vs 0.558, $P < .001$) when AMI was defined by hs-cTn compared with when AMI was defined by cTn (Table IV and online Appendix Supplementary Table IV).

When strictly applying the definition of hs-cTn measuring the 99th percentile upper reference limit with an analytical imprecision of <10%,[30,31] Keller et al[22] and Pracon et al[26] are no longer considered under the category of hs-cTn. Therefore, we repeated the previous analysis with 15 studies to determine whether this significantly affected our previous findings. When using studies that used strict hs-cTn assays, baseline cTn and hs-cTn had similar values to those before in regard to sensitivity (0.752 [0.727-0.775] vs 0.877 [0.857-0.894]), specificity (0.939 [0.933-0.946] vs 0.793 [0.782-0.803]), PPV (0.750 [0.725-0.773] vs 0.505 [0.484-0.526]), NPV (0.940 [0.933-0.946] vs 0.964 [0.958-0.969]), positive LR (10.366 [6.475-16.595] vs 4.002[3.203-4.999]), negative LR (0.259 [0.204-0.329] vs 0.164 [0.119-0.225]), diagnostic OR (44.019 [23.073-83.983] vs 28.645 [18.135-45.247]), and SROC (0.893 [0.835-0.951] vs 0.916 [0.888-0.944]). Using a strict definition of hs-cTn compared with the study-defined hs-cTn (Table III) lowered specificity (0.793 vs 0.816, respectively; $P < .01$) and PPV (0.505 vs 0.558, respectively; $P < .01$) but was not significantly associated with sensitivity, NPV, positive LR, negative LR, diagnostic OR, or area under the SROC curve.

cTn and hs-cTn for prognosis

Outcome data were provided for 10 studies only because data could not be accurately extracted from Keller et al.[22] During a mean follow-up of 12.3 months (Table I), our study demonstrated that patients with an elevated baseline cTn or elevated baseline hs-cTn have significantly higher incidence of death (online Appendix Supplementary Figure 1A), nonfatal MI (online Appendix Supplementary Figure 1B), or their combination (online Appendix Supplementary Figure 1C) compared with patients who had a negative baseline cTn or negative baseline hs-cTn, respectively. The ORs for baseline cTn and baseline hs-cTn are not significantly different for the outcomes of death (online Appendix Supplementary Figure 1A; $P = .46$, $I^2 = 0\%$), nonfatal MI (online Appendix Supplementary Figure 1B; $P = .62$, $I^2 = 0\%$), or their combination (online Appendix Supplementary Figure 1C; $P = .75$, $I^2 = 0\%$) during follow-up. However,

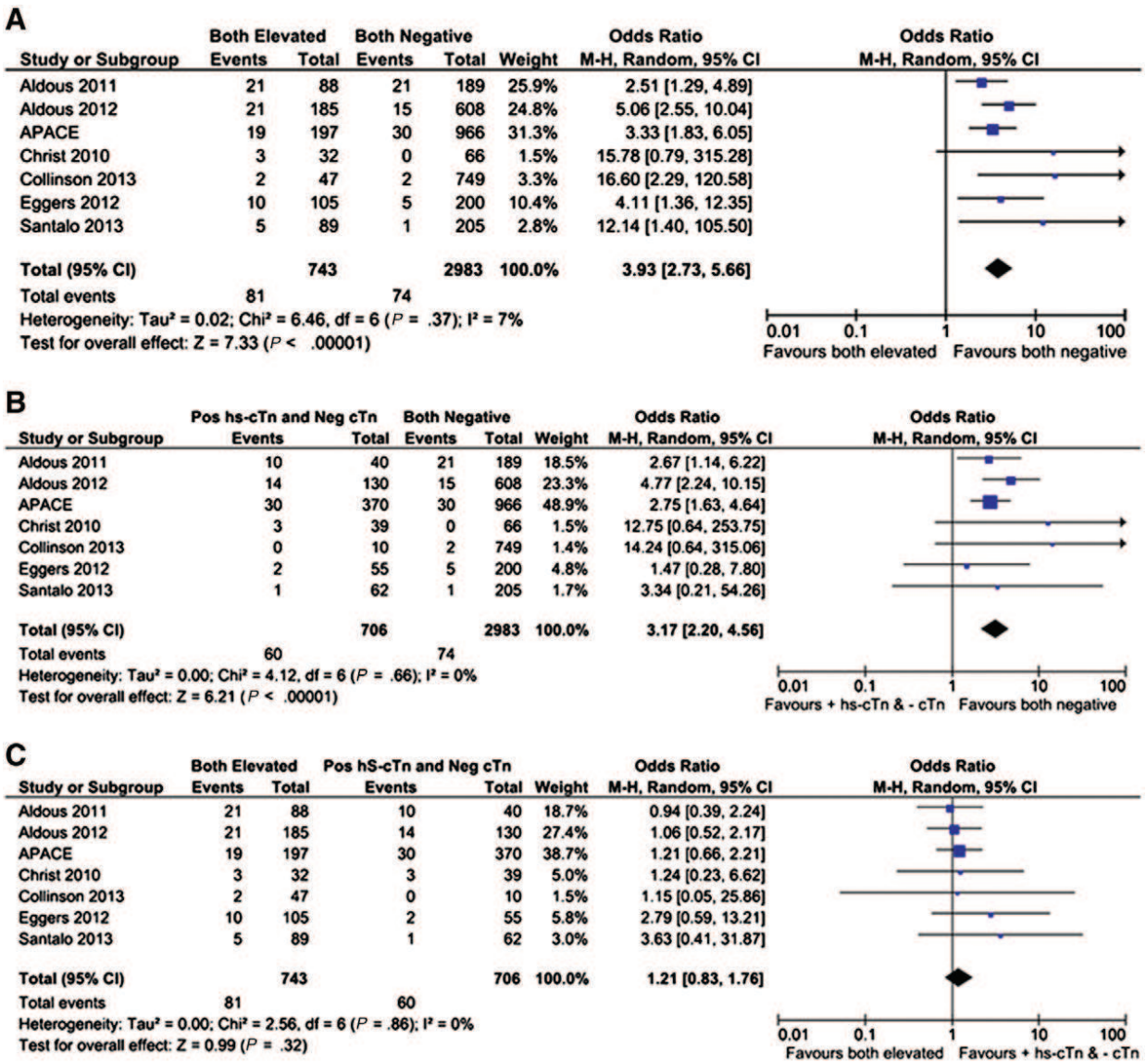
Figure 3



Forest plots comparing death during follow-up between patients with elevation of both baseline cTn and baseline hs-cTn and patients with both negative baseline cTn and baseline hs-cTn (A), death during follow-up between patients with elevation of baseline hs-cTn and negative baseline cTn and patients with both negative baseline cTn and baseline hs-cTn (B), and death during follow-up between patients with elevation of both baseline cTn and baseline hs-cTn and patients with elevation of baseline hs-cTn and negative baseline cTn (C) for patients that presented with chest pain.

significantly more individuals with an elevated baseline hs-cTn died (173 with elevated baseline hs-cTn died vs 105 with elevated baseline cTn died of the 231 total individuals who died during follow-up, $P < .001$) or developed AMI (143 with elevated baseline hs-cTn developed MI vs 92 with elevated baseline cTn developed MI of 222 total individuals who had AMI, $P < .001$) during follow-up compared with individuals with an elevated baseline cTn.

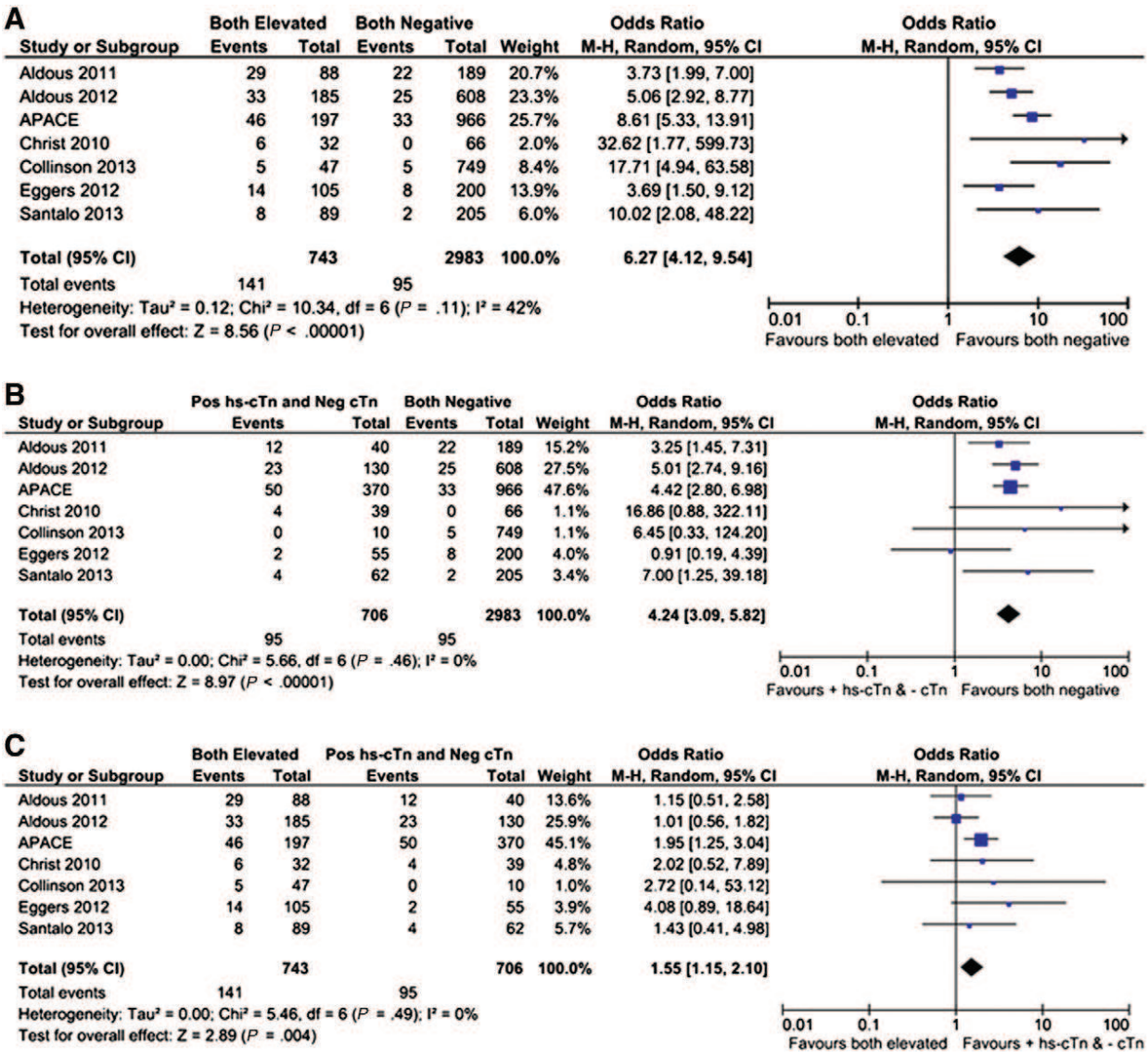
Figure 4



Forest plots comparing nonfatal MI during follow-up between patients with elevation of both baseline cTn and baseline hs-cTn and patients with both negative baseline cTn and baseline hs-cTn (A), nonfatal MI during follow-up between patients with elevation of baseline hs-cTn and negative baseline cTn and patients with both negative baseline cTn and baseline hs-cTn (B), and nonfatal MI during follow-up between patients with elevation of both baseline cTn and baseline hs-cTn and patients with elevation of baseline hs-cTn and negative baseline cTn (C) for patients that presented with chest pain.

Patients who had elevation of both baseline cTn and baseline hs-cTn had significantly greater death (Figure 3A), nonfatal MI (Figure 4A), and their combination (Figure 5A) during follow-up compared with patients with both negative baseline cTn and baseline hs-cTn. Patients who had elevation of baseline hs-cTn but a negative baseline cTn had significantly greater death (Figure 3B), nonfatal MI (Figure 4B), and their combination (Figure 5B) during follow-up compared with patients with both negative baseline cTn and baseline hs-cTn. Patients with elevation of both baseline cTn and baseline hs-cTn had significantly greater death (Figure 3C) and the combination end point of death and nonfatal MI (Figure 5C) but no significant difference in nonfatal MI (Figure 4C) during follow-up compared with patients with an elevated baseline hs-cTn but a negative baseline cTn. Visual inspection of funnel plots along with Peters test did not show evidence of publication bias for baseline cTn (Peters test, $P = .75$) and for baseline hs-cTn (Peters test, $P = .53$).

Figure 5



Forest plots comparing the combination endpoint of death and nonfatal MI during follow-up between patients with elevation of both baseline cTn and baseline hs-cTn and patients with both negative baseline cTn and baseline hs-cTn (A), combination endpoint during follow-up between patients with elevation of baseline hs-cTn and negative baseline cTn and patients with both negative baseline cTn and baseline hs-cTn (B), and combination during follow-up between patients with elevation of both baseline cTn and baseline hs-cTn and patients with elevation of baseline hs-cTn and negative baseline cTn (C) for patients that presented with chest pain.

Discussion

This systematic review and collaborative meta-analysis on 8,644 patients demonstrated that hs-cTn and cTn have excellent overall diagnostic accuracy for AMI in patients with chest pain. The hs-cTn assay has the benefit of a significantly greater sensitivity and NPV with a lower negative LR compared with cTn. However, this is at the cost of specificity, PPV, and positive LR. Meta-regression analysis also suggested that time from onset of chest pain to presentation was significantly associated with test performance for baseline cTn but was not associated with test performance accuracy for baseline hs-cTn. These data validate previous works suggesting that hs-cTn can more accurately diagnose or exclude AMI early after chest pain.[32] Prevalence of AMI, STEMI, diabetes mellitus, and male sex also was associated with test performance for baseline cTn but not baseline hs-cTn. When AMI adjudication is performed with hs-cTn as the criterion standard to define AMI, baseline hs-cTn had better test performance as assessed by pooled area under

the ROC curve compared with baseline cTn. Elevation of baseline hs-cTn identified a greater number of patients who died or had nonfatal MI during follow-up compared with elevation of baseline cTn. Finally, these data demonstrate that baseline elevation of hs-cTn but a negative baseline cTn was associated with an incremental increase in risk for death or nonfatal MI during follow-up. Although troponin assays have previously been compared in meta-analysis,[33] our meta-analysis is the first to focus specifically on diagnostic and prognostic role of hs-cTn and conventional cTn and performed meta-regression to assess the affect of different variables on diagnostic accuracy. These data support a broader acceptance of hs-cTn.

The development of a universal definition for AMI[34] has greatly aided the field of cardiology by providing a means to reliably compare diagnostic tests and therapies. Likewise, establishment of standards for cardiac troponins and adoption of common cut-points[30,31,35,36] may not only enable improved comparison between assays but also help provide uniform data that physicians can more readily and confidently apply to clinical practice. Adoption of hs-cTn into the ED evaluation of chest pain may significantly alter current practice. Although hs-cTn may enable rapid rule out of patients who present to the ED with chest pain,[32,37] concern exists that the reduction in PPV and specificity may lead to more extensive cardiovascular testing. Although minimal elevations in hs-cTn may not necessarily identify AMI, it is important to recognize that these patients are at increased risk for adverse outcomes and should receive appropriate medical intervention.[4] Finally, it is also critical to interpret these biomarkers in the clinical context of the patient. The importance of clinical history and appropriate electrocardiographic evaluation cannot be underestimated. For example, the diagnostic value of a negative troponin is less helpful if the patient's presentation is consistent with unstable angina because the clinical presentation will guide management rather than the biomarker result.

This meta-analysis has several important limitations. To enable appropriate comparison of cTn and hs-cTn in a "real-world" scenario, we excluded studies in which patients were limited to those with a baseline negative troponin because this inherently introduces bias. Similarly, we excluded studies that were limited to only patients with ACS or specific populations. We did not exclude studies with STEMI patients, although this is an electrocardiographic and clinical diagnosis, as we wished to assess the diagnostic accuracy of the assays in all patients with chest pain. The relatively high incidence of AMI in our population does lead to a bias in the PPV of the test, which is important to acknowledge. However, positive and negative LR should not be influenced by this bias. Other limitations are those inherent to meta-analyses, which include lack of raw or uniform data, and use of different troponin assays and cut-points. We were also unable to adjust the diagnosis of AMI based on the delta for the rise and/or fall of troponin and the use of longer follow-up may admix events related to ACS with those related to the predictive value of cTn detected in the absence of ACS. Although a random-effect pooling method adjusts for it, another limitation of this meta-analysis is the heterogeneity observed among studies, although this appeared to be low. Finally, meta-regression techniques are limited given the lack of raw patient information and should therefore be viewed with caution and as hypothesis generating.

In conclusion, both cTn and hs-cTn have excellent diagnostic accuracy, but our data support broader use of hs-cTn given the improvements provided in sensitivity, NPV, and identification of patients at risk for adverse outcomes during follow-up.

Conflicts of Interest/Disclosures

M.J.L., N.C.B., R.O.E., R. Torguson, F.C., S.J.A., S.W.G., K.I., M.S., J.P.C., Y.F., R. Twerenbold, R.W.: none; M.C.: research support and speaker's honoraria from Roche Diagnostics; P.O.C.: consultant for Philips Health Care Incubator and Siemens Point of Care; J.M.: consultant for Philips Health Care Incubator; U.L.: study fees from St Jude Medical and Medtronic, lecture honoraria from St Jude Medical, Medtronic, Sanofi, Aventis, Boehringer Ingelheim, and Bristol:Myers Squibb; C.C.G.: honoraria from Brahms Thermofisher; C. Meune: grant support from Roche Diagnostics and Brahms Thermofisher, and lecture fees from Roche Diagnostics; K.M.E.: honoraria from Siemens Healthcare Diagnostics and consultant for Abbott Laboratories and Fiomi Diagnostics; R.P.: research grant from Abbott Diagnostics; DHS: research grant from Abbott Laboratories and Singulex, Inc; A.H.B.W.: research grant from Singulex, Inc, Roche Laboratories, Alere, and Beckman Coulter, and travel support from Abbott Laboratories; J.O.L.: research support and consultant honoraria from Abbott Diagnostics, Alere, and Roche Diagnostics; A.S.J.: consultant for Roche Laboratories, Radiometer, Abbott Laboratories, Alere Criticical Diagnostics, Ortho Diagnostics, Beckman Coulter, and Amgen; C. Mueller: research support from the European Union, Swiss National Science Foundation, Swiss Heart Foundation, Basel University, University Hospital Basel, Cardiovascular Research Foundation Basel, Stanley Thomas Johnson Foundation, Abbott, ALERE, Beckman Coulter, Brahms, Bühlmann, Critical Diagnostics, Nanosphere, Pronota, Roche, Siemens, and 8sense, and speaker or consulting honoraria from Abbott, ALERE, BG Medicine, Bio Merieux, Brahms, Massachusetts General Hospital, Novartis, Roche, and Siemens.

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Appendix

Supplementary Table I. Appraisal of included studies

Study	Standard troponin assays	Prespecified cut-points	Study design	Consecutive patient inclusion	Withdrawals reported	AMI definition	Troponin used to define AMI
Aldous et al 2012	Yes	Yes	Prospective	No	Yes	Universal definition with physician adjudication. Biomarker elevation with a rise or fall or signs of CAD	Conventional
Aldous et al 2011	Yes	Yes	Prospective	No	Yes	Universal definition with physician adjudication. Biomarker elevation with a 20% rise or fall or signs of CAD	Conventional
APACE	Yes	Yes	Prospective	Yes	Yes	Universal definition with physician adjudication. Biomarker elevation with a 30% rise or fall or signs CAD	Conventional
Christ et al	Yes	Yes	Retrospective	Yes	Yes	Universal definition with physician adjudication. Biomarker elevation with a 20% rise or fall or signs of CAD	Conventional
Collinson et al	Yes	Yes	Prospective	No	Yes	Universal definition with physician adjudication. Biomarker elevation with a rise or fall or signs of CAD	Conventional
Eggers et al	Yes	Yes	Prospective	Yes	No	Universal definition with physician adjudication Biomarker elevation with a 20% rise or fall, an absolute change of ≥5 ng/L, or signs of CAD	Conventional
Freund et al	Yes	Yes	Prospective	Yes	Yes	Universal definition with physician adjudication. Biomarker elevation with symptoms or signs of CAD	Conventional
Hammerer-Lercher et al	Yes	Yes	Retrospective	Yes	Yes	Universal definition with physician adjudication. Biomarker elevation with a rise or fall or signs of CAD	Conventional
Inoue et al	Yes	Yes	Prospective	Yes	Yes	Universal definition with physician adjudication.	Conventional
Keller et al	Yes	Yes	Prospective	Yes	Yes	Universal definition with physician adjudication. Biomarker elevation with a 20% rise or fall or signs of CAD	Conventional
Lotze et al	Yes	Yes	Prospective	Yes	Yes	Universal definition with physician adjudication. Biomarker elevation with a rise or fall or signs of CAD	Combination
Melki et al	Yes	Yes	Prospective	No	Yes	Universal definition with physician adjudication. Biomarker elevation with a rise or fall or signs of CAD	Conventional
Meune et al	Yes	Yes	Prospective	Yes	Yes	Universal definition with physician adjudication. Biomarker elevation with a rise or fall	Conventional
Pracon et al	Yes	Yes	Prospective	Yes	Yes	Universal definition with physician adjudication. Biomarker elevation with a rise or fall or signs of CAD	Combination
Santalo et al	Yes	Yes	Prospective	Yes	Yes	Universal definition with physician adjudication. Biomarker elevation with a 20% rise or fall	Conventional
Schreiber et al	Yes	Yes	Prospective	No	Yes	Universal definition with physician adjudication. Biomarker elevation with a rise or fall	Combination
Sebbane et al	Yes	Yes	Prospective	Yes	Yes	Universal definition with physician adjudication. Biomarker elevation with a rise or fall or signs of CAD	Conventional

Abbreviation: *CAD*, Coronary artery disease.

Supplementary Table II. Number of TPs, FPs, FNs, and TNs based on the baseline cTn at presentation or baseline hs-cTn at presentation cut-point and whether the patient experienced AMI

		cTn							
Study	**Conventional cTn cut-point**	**TP (n)**	**FP (n)**	**FN (n)**	**TN (n)**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
Aldous et al 2012	Abbott Architect cTnI, 30 ng/L	175	26	30	708	85.4	96.5	87.1	95.9
Aldous et al 2011	Abbott Architect cTnI, 30 ng/L	82	21	28	201	74.5	90.5	79.6	87.8
APACE	Roche cTnT 4th gen, 35 ng/L	168	29	66	1270	71.8	97.8	85.3	95.1
Christ et al	Roche cTnT 4th gen, 35 ng/L	13	11	7	106	65.0	90.6	54.2	93.8
Collinson et al	Siemens Stratus CS cTnI, 70 ng/L	53	29	10	739	84.1	96.2	64.6	98.7
Eggers et al	Siemens Stratus CS cTnI, 70 ng/L	92	13	36	219	71.9	94.4	87.6	85.9
Freund et al	Siemens Xpand HM cTnI, 140 ng/L or Beckman Coulter Access cTnI, 60 ng/L	32	9	13	263	71.1	96.7	78.0	95.3
Hammerer-Lercher et al	Roche Elecsys cTnT 4th gen, 10 ng/L	35	48	5	352	87.5	88.0	42.2	98.6
Inoue et al	Roche cTnT 4th gen, 35 ng/L	98	33	65	87	60.1	72.5	74.8	57.2
Keller et al	Roche Elecsys cTnT 4th gen, 10 ng/L	300	83	113	1322	72.6	94.1	78.3	92.1
Lotze et al	Roche cTnT 4th gen, 100 ng/L	11	38	2	91	84.6	70.5	22.4	97.8
Melki et al	Roche cTnT 4th gen, 40 ng/L	90	7	24	112	79.0	94.1	92.8	82.4
Meune et al	Siemens Xpand HM cTnI, 70 ng/L	12	7	1	38	92.3	84.4	63.2	97.4
Pracon et al	Siemens Dimension Flex TnI, 70 mg/L	69	20	15	83	82.1	80.6	77.5	84.7
Santalo et al	Roche Cobas e401 cTnT 4th gen, 10 ng/L	61	28	17	250	78.2	89.9	68.5	93.6
Schreiber et al	Siemens Dimension RxL TnI, 140 ng/L	9	14	3	439	75.0	96.9	39.1	99.3
Sebbane et al	Beckman Access2 cTnI, 40 ng/L	38	10	13	133	74.5	93.0	79.2	91.1

		hs-cTn							
Study	hs-cTn cut-point	TP (n)	FP (n)	FN (n)	TN (n)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Aldous et al 2012	Roche HS TnT, 14 ng/L	181	134	24	600	88.3	81.7	57.5	96.2
Aldous et al 2011	Roche HS TnT, 14 ng/L	92	36	18	186	83.6	83.8	71.9	91.2
APACE	Roche HS TnT, 14 ng/L	221	346	13	953	94.4	73.4	39.0	98.7
Christ et al	Roche HS TnT, 14 ng/L	19	45	1	72	95.0	61.5	29.7	98.6
Collinson et al	Beckman AccuTnI, 40 ng/L	43	15	20	757	68.2	98.1	74.1	97.4
Eggers et al	Roche HS TnT, 14 ng/L	101	59	27	173	78.9	74.6	63.1	86.5
Freund et al	Roche HS TnT, 14 ng/L	42	48	3	224	93.3	82.4	46.7	98.7
Hammerer-Lercher et al	Roche HS TnT, 14 ng/L	36	80	4	320	90.0	80.0	31.0	98.8
Inoue et al	Roche HS TnT, 14 ng/L	141	59	22	61	86.5	50.8	70.5	73.5
Keller et al	Siemens sensitive TnI Ultra, 40 ng/L	375	138	38	1267	90.8	90.2	73.1	97.1
Lotze et al	Roche HS TnT, 14 ng/L	12	60	1	69	92.3	53.5	16.7	98.6
Melki et al	Roche HS TnT, 14 ng/L	111	31	3	88	97.4	73.9	78.2	96.7
Meune et al	Roche HS TnT, 14 ng/L	12	12	1	33	92.3	73.3	50.0	97.1
Pracon et al	Abbott Architect Stat TnI, 28 ng/L	73	12	11	91	86.9	88.3	85.9	89.2
Santalo et al	Roche HS TnT, 14 ng/L	70	79	8	199	89.7	71.6	47.0	96.1
Schreiber et al	Singulex Erenna HS-TnI, 8 ng/L	10	80	2	373	83.3	82.3	11.1	99.5
Sebbane et al	Roche HS TnT, 14 ng/L	39	22	12	121	76.5	84.6	63.9	91.0

Abbreviations: *TP*, True positive; *FP*, false positive; *FN*, false negative; *TN*, true negative.
Abbott (Abbott Park, IL), Roche (Indianapolis, IN), Siemens (Tarrytown, NY), Singulex (St Louis, MO).

Supplementary Table III. Number of TPs, FPs, FNs, and TNs based on the second cTn or second hs-cTn cut-point and whether the patient experienced AMI for studies providing this data

Study	Time since presentation (h)	TP (n)	FP (n)	FN (n)	TN (n)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
cTn at second serial blood sampling									
Aldous et al 2012	2	189	30	16	704	92.2	95.9	86.3	97.8
Aldous et al 2011	6	100	26	10	196	90.9	88.3	79.4	95.1
APACE	2	88	23	11	788	88.9	97.2	79.3	98.6
Christ et al	6	13	12	7	105	65.0	89.7	52.0	93.8
Collinson et al	1.5	13	12	1	643	92.9	98.2	52.0	99.8
Freund et al	6	9	2	1	107	90.0	98.2	81.8	99.1
Meune et al	3	11	9	2	36	84.6	80.0	55.0	94.7
Pracon et al	4	20	8	2	22	90.9	73.3	71.4	91.7
Santalo et al	2	63	28	8	239	88.7	89.5	69.2	96.8
Schreiber et al	1.5	7	14	2	384	77.8	96.5	33.3	99.5
hs-cTn at second serial blood sampling									
Aldous et al 2012	2	189	149	16	585	92.2	79.7	55.9	97.3
Aldous et al 2011	6	100	41	10	181	90.9	81.5	70.9	94.8
APACE	2	96	231	2	579	98.0	71.5	29.4	99.7
Christ et al	4	15	41	5	76	75.0	65.0	26.8	93.8
Collinson et al	1.5	9	7	2	647	81.8	98.9	56.3	99.7
Freund et al	6	5	12	0	44	100.0	78.6	29.4	100.0
Meune et al	3	13	14	0	31	100.0	68.9	48.1	100.0
Pracon et al	3	11	2	1	16	91.7	88.9	84.6	94.1
Santalo et al	2	65	75	4	213	94.2	74.0	46.4	98.2
Schreiber et al	1.5	9	72	0	326	100.0	81.9	11.1	100.0

Abbreviations: *TP*, True positive; *FP*, false positive; *FN*, false negative; *TN*, true negative.

Supplementary Table IV. Number of TPs, FPs, FNs, and TNs based on the baseline cTn or baseline hs-cTn cut-point and whether the patient experienced AMI when AMI was defined using the cut-point for the hs-cTn assay

Study	TP (n)	FP (n)	FN (n)	TN (n)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Baseline cTn								
Aldous et al 2011	105	26	24	177	81.4	87.2	80.2	88.1
APACE	163	20	149	1201	52.2	98.4	89.1	89.0
Christ et al	13	11	22	91	37.1	89.2	54.2	80.5
Collinson et al	54	28	12	737	81.8	96.3	65.9	98.4
Melki et al	92	9	39	93	70.2	91.2	91.1	70.5
Santalo et al	89	62	13	192	87.3	75.6	58.9	93.7
Baseline hs-cTn								
Aldous et al 2011	116	14	13	189	89.9	93.1	89.2	93.6
APACE	281	286	31	935	90.1	76.6	49.6	96.8
Christ et al	33	31	2	71	94.3	69.6	51.6	97.3
Collinson et al	45	13	21	756	68.2	98.3	77.6	97.3
Melki et al	128	18	3	84	97.7	82.4	87.7	96.6
Santalo et al	61	25	41	237	59.8	90.5	70.9	85.3

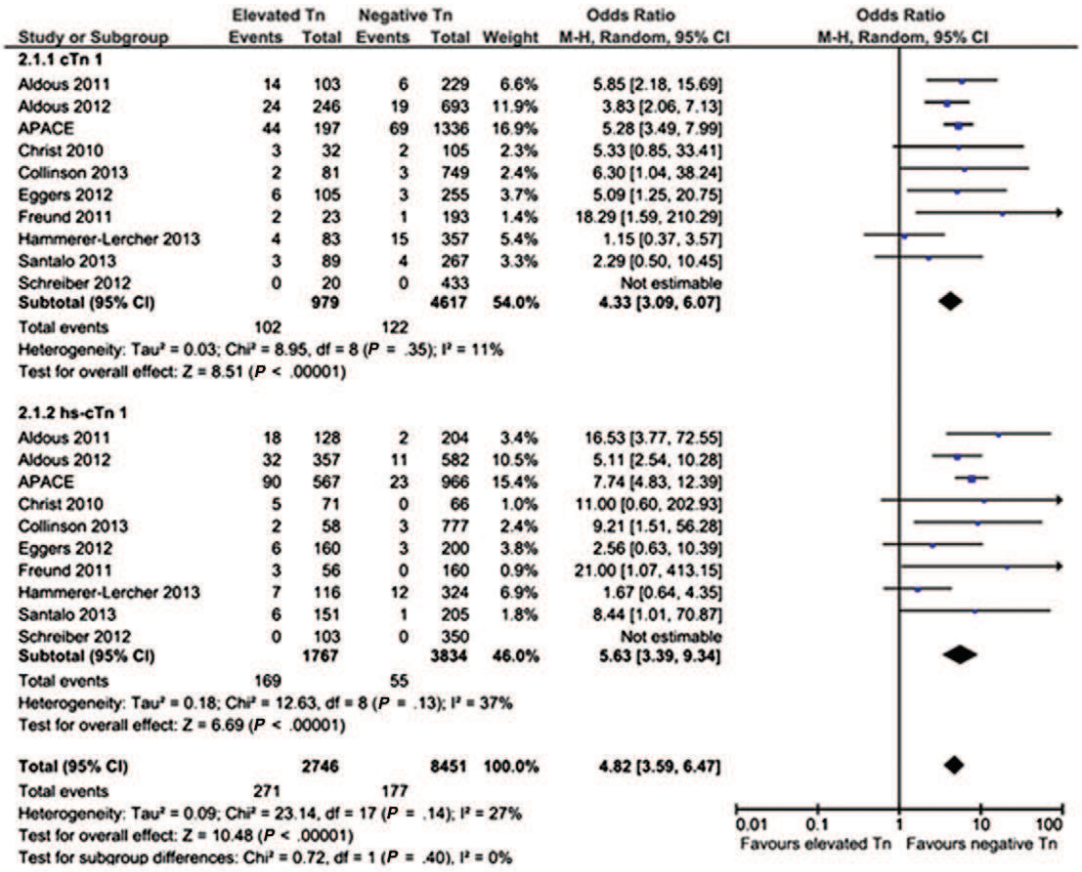
Abbreviations: *TP*, true positive; *FP*, false positive; *FN*, false negative; *TN*, true negative.

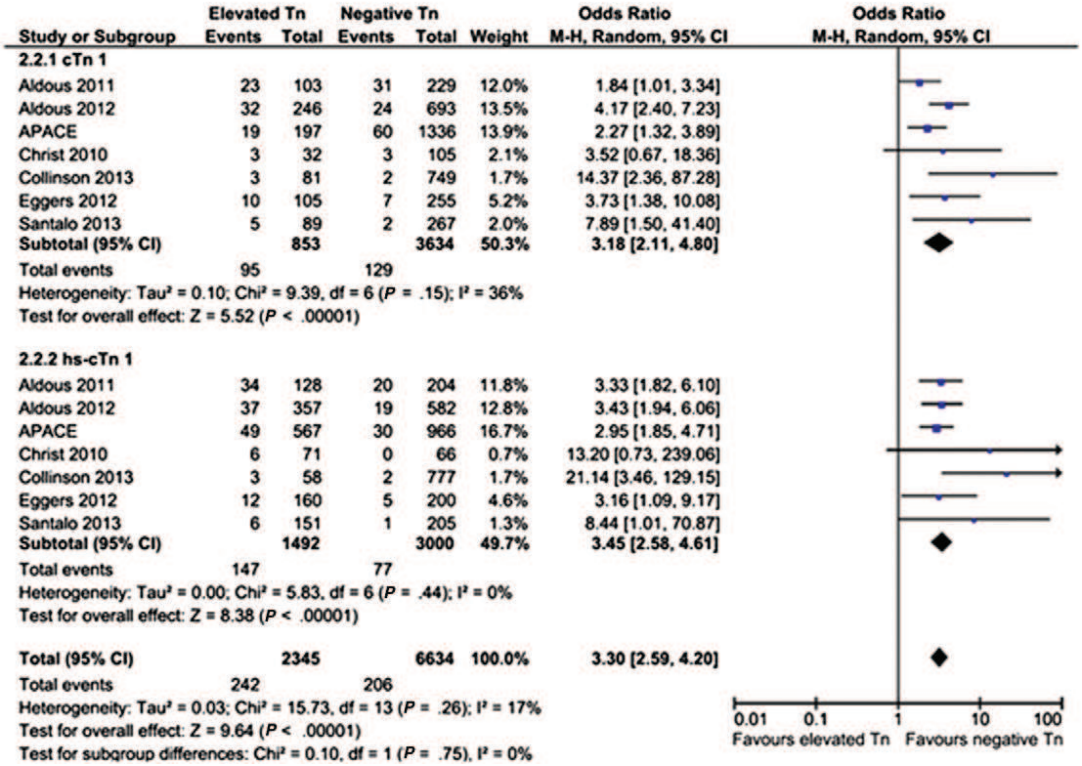
Supplementary Table V. Area under the ROC curves for the admission and second conventional and hs-cTn for the diagnosis of AMI

Study	Conventional cTn, AUC ± SE	Hs-cTn, AUC ± SE	Time to next troponin (h)	Next conventional cTn, AUC ± SE	Next Hs-cTn, AUC ± SE
Aldous et al 2012	0.96 ± 0.01	0.92 ± 0.01	2	0.98 ± 0.01	0.93 ± 0.01
Aldous et al 2011	0.88 ± 0.02	0.90 ± 0.02	6	0.93 ± 0.02	0.94 ± 0.02
APACE	0.79 ± 0.06	0.92 ± 0.02	2	0.97 ± 0.02	0.97 ± 0.01
Christ et al	0.89 ± 0.04	0.91 ± 0.03	6	0.97 ± 0.02	0.97 ± 0.01
Collinson et al	0.94 ± 0.02	0.92 ± 0.02	1.5	0.95 ± 0.05	0.94 ± 0.06
Eggers et al	0.91 ± 0.02	0.85 ± 0.02		NR	NR
Freund et al	0.93 ± 0.02	0.93 ± 0.02	6	0.85 ± 0.10	0.94 ± 0.05
Hammerer-Lercher et al	0.91 ± 0.02	0.94 ± 0.01		NR	NR
Inoue et al	0.68 ± 0.03	0.73 ± 0.03		NR	NR
Keller et al	0.85 ± 0.02	0.96 ± 0.02	3	0.98 ± 0.01	0.98 ± 0.01
Lotze et al	0.85 ± 0.03	0.87 ± 0.03		NR	NR
Melki et al	0.93 ± 0.02	0.95 ± 0.02	2	0.96 ± 0.01	0.96 ± 0.01
Meune et al	0.95 ± 0.05	0.92 ± 0.04	3	0.98 ± 0.02	0.97 ± 0.02
Pracon et al	0.86 ± 0.03	0.92 ± 0.02	4	0.86 ± 0.05	0.91 ± 0.06
Santalo et al	0.83 ± 0.12	0.81 ± 0.10	2	0.96 ± 0.04	0.84 ± 0.09
Schreiber et al	0.90 ± 0.01	0.94 ± 0.01	1.5	0.87 ± 0.02	0.98 ± 0.01
Sebbane et al	0.90 ± 0.03	0.89 ± 0.02		NR	NR

Abbreviations: *AUC*, Area under the ROC curve; *NR*, not reported.

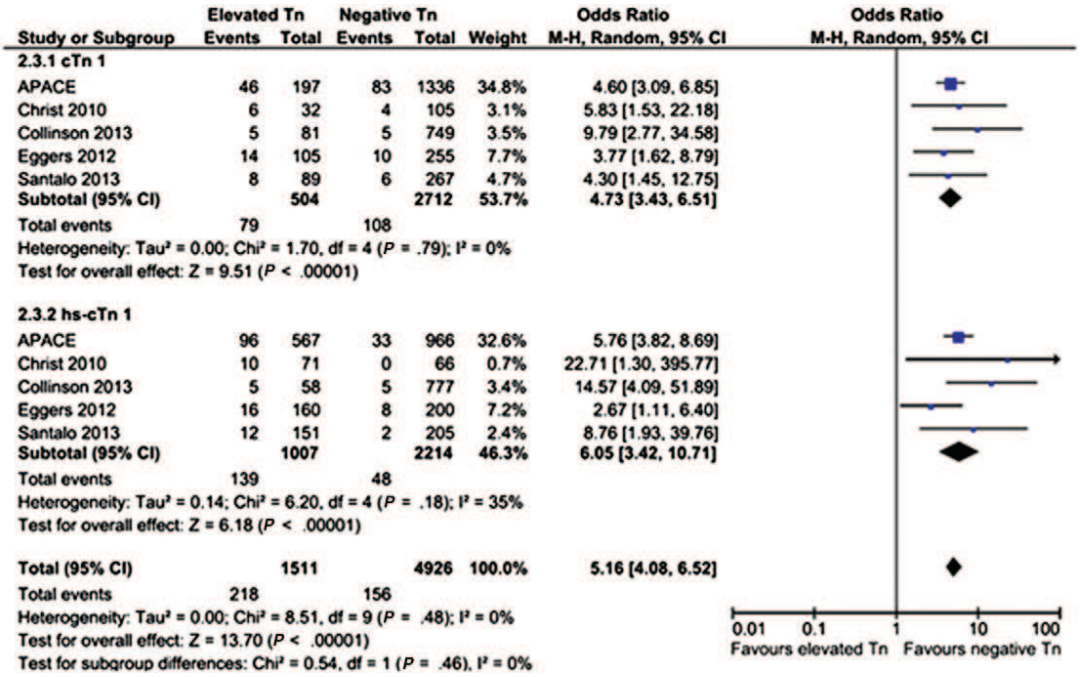
Supplementary Figure 1





Forest plots comparing death (A), nonfatal MI (B), or their combination (C) for patients that presented with chest pain stratified based on whether or not they had an elevated baseline cTn or baseline hs-cTn level or a negative troponin level.

Supplementary Figure 1



(Continued.)

Appendice 5 : Influence de l'âge et de la fonction rénale sur les performances de l'HsTnT

Influence of Age and Renal Function on High-Sensitivity Cardiac Troponin T Diagnostic Accuracy for the Diagnosis of Acute Myocardial Infarction

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Concerns have been raised about the performance of highly sensitive cardiac troponin assays to accurately detect acute myocardial infarction (AMI), particularly in non−ST segment elevation (NSTEMI), in elderly patients, and in patients with renal failure. We evaluated whether increased age and low estimated glomerular filtration rate (eGFR) alter diagnostic performance of high-sensitivity cardiac troponin T (HScTnT). In a prospective multicentric study, HScTnT levels were measured blindly at presentation in patients with acute chest pain. Three hundred and sixty-seven patients were enrolled, including 84 patients ≥70 years. Final diagnosis was AMI for 57 patients (16%) and NSTEMI for 43 patients (12%). NSTEMI was more frequent in elderly patients (p = 0.008). Sensitivity and specificity of HScTnT >14 ng/L at admission for AMI were 96% and 51% in patients ≥70 years versus 91% (NS) and 88% (p <0.0001) in younger patients; the same observations were done for the diagnosis of NSTEMI. Given an HScTnT >53.5 ng/L for the diagnosis of AMI and NSTEMI, respective sensitivities were 87% and 84% and respective specificities were 87% and 87% in elderly patients. Using a cutoff at 35.8 ng/L (for AMI) or 43.2 ng/L (for NSTEMI), sensitivities were 94% and 92%, and specificities were 86% and 88% in patients with low eGFR. Older age, but not low eGFR, was an independent predictive factor of an elevated HScTnT at admission (odds ratio 2.2 [1.2−3.9], p = 0.007). In conclusion, adapted thresholds of HScTnT are required for an accurate diagnosis of AMI/NSTEMI in patients aged ≥70 and in those with low eGFR. **(Am J Cardiol 2013;111:1701−1707)**

Cardiac troponins (cTn, either the T or I isoform) are the preferred biomarkers measured in patients with suspected acute myocardial infarction (AMI).[1,2] The recently available high-sensitivity assays for cTn (HScTn) have been demonstrated to improve the detection of AMI.[3−9] Concerns have risen about the exact performance of HScTn assays in elderly patients or in patients with renal failure. In fact, non-AMI elevations of HScTn were noted among these specific populations.[10] The uncertainty regarding the appropriate management of these patients has contributed substantially to the reluctance to use HScTn assays in clinical practice.[11] Elderly patients are prevalent among those presenting to the emergency department with chest pain.[12] Elevations of cTn were found in subjects aged >70 years,[13−15] and HScTn were shown to be correlated with age.[10,16] Furthermore, renal dysfunction may influence cTn concentrations,[17] and renal insufficiency rises with age.[18] Elevated HScTn in elderly patients without AMI may increase unnecessary hospitalizations, procedures, and iatrogenesis.[18] This study sought to determine the impact of age and renal function on the diagnostic performance of the HScTnT in the detection of AMI.

Methods

This was a post hoc analysis of 2 previous studies.[8,9] The study population consisted of patients from 2 prospective clinical evaluations of HScTnT testing.[8,9] The study was performed in 3 centers in the Paris area. We prospectively enrolled patients (>18 years) presenting to the emergency department[8] or to the cardiology unit[9] with a suspected diagnosis of AMI (chest pain onset <6 hours). Patients requiring renal replacement therapy were excluded. The study complied with the principles of the Declaration of

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Author contributions: PR, CCG, and CM designed the study. BD, YEC, KW, SZ, CM, and PR helped collecting the data. CCG carried out the statistical analysis and the biochemical assays. CCG, YF, and PR prepared the manuscript. BR, CM, YEC corrected the manuscript.

See page 1706 for disclosure information.

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Table 1
Baseline characteristics of the population according to age

Variable	All patients (n = 367)	Age(Yrs)		p Value*
		≥70 (n = 84)	<70 (n = 283)	
Age (yrs)	57 ± 16	81 ± 8	50 ± 11	—
Men	237 (65%)	43 (51%)	194 (69%)	0.005
Familial history of CAD	118 (32%)	21 (25%)	97 (34%)	0.160
History of CAD	102 (28%)	40 (48%)	62 (22%)	<0.0001
Dyslipidemia†	135 (37%)	34 (40%)	101 (36%)	0.455
Smoker	147 (40%)	21 (25%)	126 (45%)	0.003
Diabetes mellitus	56 (15%)	35 (42%)	21 (7%)	0.008
Hypertension	138 (38%)	51 (61%)	87 (31%)	<0.0001
Prior heart failure	25 (7%)	18 (21%)	7 (2%)	<0.0001
Typical thoracic pain	166 (45%)	34 (40%)	132 (47%)	0.383
Coronarography	128 (35%)	26 (31%)	102 (36%)	0.453
Aspirin	137 (37%)	35 (42%)	102 (36%)	0.419
Clopidogrel	62 (17%)	14 (17%)	48 (17%)	0.918
Hospital-admission	246 (67%)	64 (76%)	182 (64%)	0.074
Admission in ICU	191 (52%)	43 (51%)	148 (52%)	0.957
At admission, patients with				
cTnI >10% CV value	54 (15%)	26 (31%)	28 (10%)	<0.0001
HS-cTnT >99th percentile‡	114 (31%)	52 (62%)	62 (22%)	<0.0001
Median eGFR (ml/min per 1.73 m^2)	75.3 (62.7−91.7)	60.8 (46.6−71.3)	80.5 (67.4−95.8)	<0.001
Final AMI diagnosis	57 (16%)	23 (27%)	34 (12%)	0.001
STEMI	14 (4%)	4 (5%)	10 (4%)	0.848
NSTEMI	43 (12%)	19 (23%)	24 (8%)	0.008
Final UA diagnosis	26 (7%)	7 (8%)	19 (7%)	0.790
Other diagnosis§	284 (77%)	54 (64%)	230 (81%)	0.284

Results are in mean ± SD, median (25th−75th percentile) or n (%).
CAD = coronary acute disease; ICU = intensive care unit; LMWH = low molecular weight heparin.
* Between patients aged ≥70 and <70 yrs.
† Hypercholesterolemia ± hypertriglyceridemia.
‡ HScTnT >14 ng/L.
§ Including stable angina (n = 24), pulmonary embolism (n = 16), myopericarditis (n = 43), heart failure (n = 6), and others.

Helsinki. The protocol was approved by local ethical committees, and all patients gave informed consent before inclusion. Recommendations of the Standards for Reporting of Diagnostic Accuracy (STARD) initiative were applied.[19] Elderly patients were defined as those aged ≥70 years.

As part of the routine assessment, all patients underwent an initial clinical evaluation, 18-lead electrocardiogram (ECG), pulse oximetry, routine blood tests, and chest x-ray. Conventional cTnI was measured at presentation and, if needed, repeated after 6 to 9 h. Plasmatic cTnI concentrations were measured on an X-pand HM analyser (Siemens Healthcare Diagnostics, Newark, NJ; Limit of detection = 0.04 μg/L, limit of quantification = 0.14 μg/L, 99th percentile value = 0.07 μg/L) in Cochin and La Pitié Salpêtrière Hospitals, and on an Access analyser (Beckman Coulter, Brea, CA; Limit of detection = 0.01 μg/L, limit of quantifications = 0.06 μg/L, 99^{th} percentile value = 0.04 μg/L) in Bicêtre Hospital; limits of quantification (10% coefficient of variation values [10% CV]) were used as the cutoff for diagnosis. The decision whether to admit the patient to the hospital or to discharge the patient, as well as medical therapy and the decision to perform coronary angiogram, was at the discretion of the physicians in charge of the patient. Attending emergency physicians and cardiologists were blinded to HScTnT results, and biologists were blinded to the suspected diagnosis.

The final diagnosis was adjudicated in all patients by 2 independent experts (1 emergency physician and 1 cardiologist) and was based on all medical records (but not HScTnT concentrations) available from presentation to 30-day follow-up. In case of disagreement, cases were reviewed and adjudicated in conjunction with a third expert. AMI was diagnosed according to current guidelines.[2] Diagnosis of AMI, either non−ST elevation (NSTEMI) or ST elevation myocardial infarction (STEMI), required a cTnI increase above the 10% CV value, associated with ≥1 of the following: symptoms of ischemia, new ST-T changes or a new Q wave on the ECG, and imaging showing new loss of viable myocardium. Unstable angina (UA) was diagnosed in presence of (1) clinical manifestations suggestive of myocardial ischemia, (2) cTnI <10% CV, and (3) an ECG indicative of ongoing ischemia, or a >70% stenosis of an epicardial coronary artery (or >50% of the left main trunk) on coronary angiography, or coronary vasospasm provoked during angiography. Additional predefined diagnostic categories included cardiac but not coronary symptoms (e.g., pericarditis or myocarditis or tachyarrhythmia), noncardiac causes, and symptoms of unknown origin.

Blood samples obtained from the cTnI measurement in routine assessment were collected in heparinized containers. Plasma HScTnT concentrations were measured on an Elecsys 2010 analyzer using the HScTnT 1-step

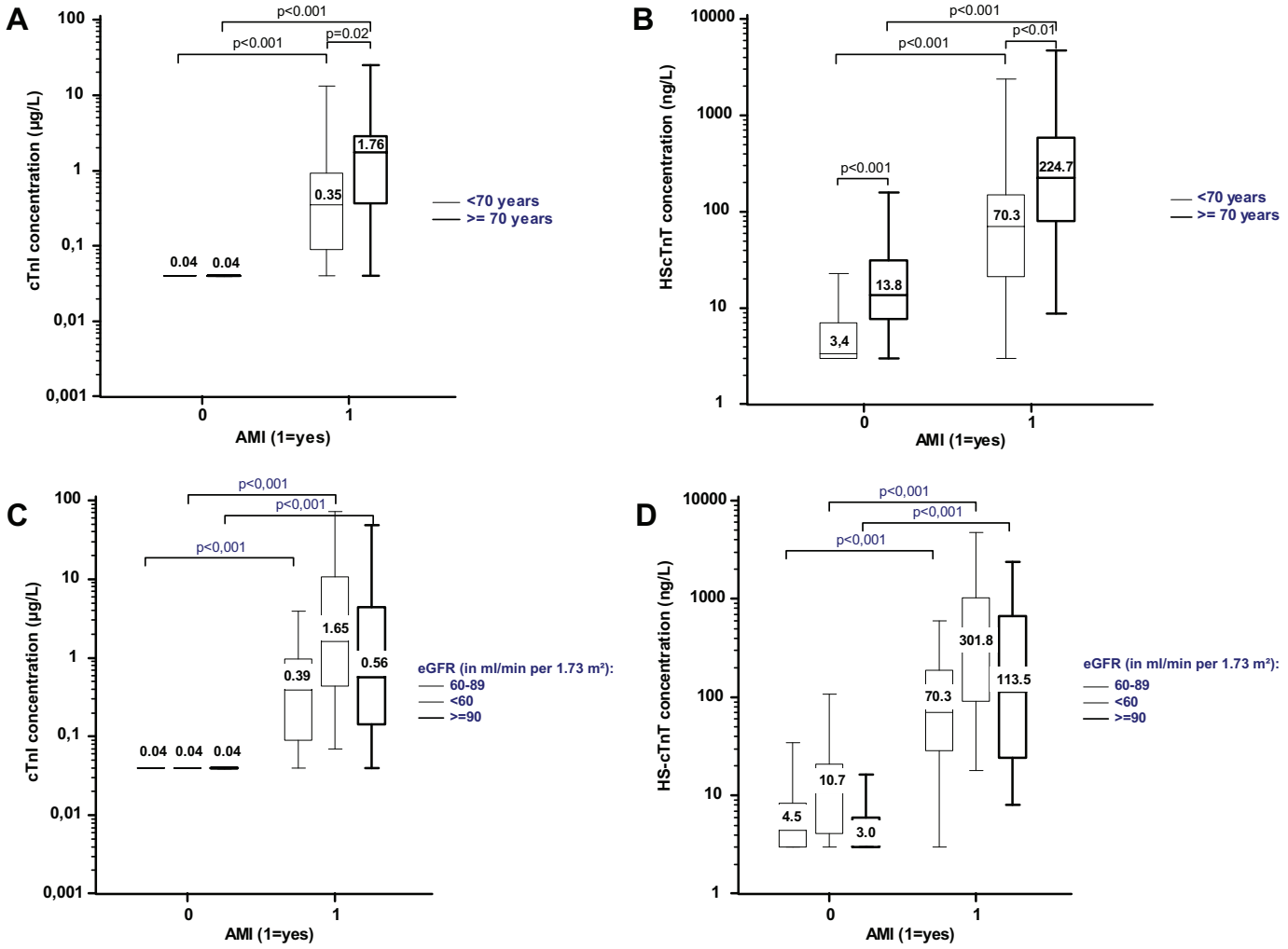

Figure 1. Values of cTnI (*A*, *C*; in µg/L) and HScTnT (*B*, *D*; in ng/L) according to final diagnosis of AMI, as a function of age (*A*, *B*) and of eGFR tertiles (*C*, *D*). cTnI values <0.04 µg/L were considered as 0.04 µg/L; HScTnT values <3 ng/L were considered as 3 ng/L.

electrochemiluminescence immunoassay (Roche Diagnostics, Meylan, France). The measuring range extended from 3 to 10,000 ng/L. The threshold for this method is 14 ng/L and corresponds to the 99th percentile. In our laboratory, the CV was <10% at 14 ng/L, and CVs obtained in Roche quality controls containing 27 and 2,360 ng/L of HScTnT were <4 %.

Plasma creatinine levels were assayed using isotope dilution mass spectrometry−standardized methods in all centers. Creatinine results were used for calculation of estimated glomerular filtration rate values (eGFR) using the revised Modification of Diet in Renal Disease formula.[20,21] Patients were classified according to chronic kidney disease stages: <60 (n = 75), 60 to 89 (n = 187), and ≥90 ml/min per 1.73 m^2 (n = 105; discussed subsequently); eGFR values ≤60 ml/min per 1.73 m^2 were indicative of kidney dysfunction.[20]

Variables are presented as mean ± SD or median (25−75 interquartile range), numbers, and percentages and compared with the Mann-Whitney U test, Pearson chi-squares test, or Fisher exact test as indicated. The Kruskal-Wallis test was used for multiple comparison (between eGFR tertiles). Correlations among variables were assessed using the Spearman coefficient. Receiver operator characteristic (ROC) curves were constructed to assess the sensitivity and specificity and positive and negative predictive values; these values are presented with their 95% confidence interval [95% CI]. Comparison of areas under the ROC curves (AUC) was performed.[22] Because of the possible impact of sample size on threshold value, ROC analysis was complemented with a bootstrap analysis (5,000 random samples with replacement) to obtain a calculation of the optimal threshold of HScTnT and its 95% confidence interval ([95%CI]).[22] A forward logistic regression was performed to assess variables associated with a positive HScTnT. Only variables with p value <0.10 in the univariate analysis were included in the logistic regression. All hypothesis testing was 2-tailed, and a p value of <0.05 was considered significant. Statistical analysis and graphs were performed using MedCalc (Medcalc software, Mariarkerke, Belgium) and R software (www.r-project.org).

Results

Three hundred and seventy-five patients were eligible for entry to the study. Creatinine results were not available in 8 patients. Results are therefore presented for 367 patients, including 84 (23%) elderly patients (Table 1). When compared with younger patients, elderly patients had

Table 2
Diagnostic accuracy of HScTnT for the diagnosis of AMI, according to age and eGFR category

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
In patients <70 years (n = 283, AMI = 34)				
HScTnT >14.0 ng/L	91 [75−98]	88 [83−91]	50 [37−63]	99 [96−100]
In patients ≥70 years (n = 84, n AMI = 23)				
HScTnT >14.0 ng/L	96 [76−100]	51 [38−64]*	42 [29−57]	97 [82−100]
HScTnT >32.4 ng/L	96 [76−100]	77 [64−86]†	61 [44−76]	98 [88−100]
HScTnT >53.5 ng/L	87 [65−97]	87 [75−94]	71 [51−86]	95 [85−99]
In patients with eGFR >60 ml/min per 1.73 m^2 (n = 291, n AMI = 41)				
HScTnT >14.0 ng/L	90 [76−97]	86 [81−90]	51 [39−63]	98 [95−99]
In patients with eGFR ≤60 ml/min per 1.73 m^2 (n = 75, n AMI = 16)				
HScTnT >14.0 ng/L	100 [76−100]	54 [40−67]‡	37 [23−53]	100 [86−100]
HScTnT >35.8 ng/L	94 [68−100]	86 [74−94]	65 [43−83]	98 [89−100]

AMI = acute myocardial infarction; eGFR = estimated glomerular filtration rate; HScTnT = high-sensitivity cardiac troponin T; NPV = negative predictive value; PPV = positive predictive value.
* p value <0.001 versus patients aged <70.
† p value = 0.057 versus patients aged <70.
‡ p value <0.001 versus patients with eGFR >60 ml/min per 1.73 m^2.

Table 3
Diagnostic accuracy of HScTnT for the diagnosis of NSTEMI, according to age and eGFR category

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
In patients <70 years (n = 273, STEMI = 24)				
HScTnT >14.0 ng/L	96 [79−100]	89 [85−93]	43 [30−57]	100 [97−100]
In patients ≥70 years (n = 80, STEMI = 19)				
HScTnT >14.0 ng/L	95 [72−100]	51 [38−64]*	38 [25−53]	97 [82−100]
HScTnT >53.5 ng/L	84 [60−97]	87 [76−94]	67 [45−84]	95 [85−99]
In patients with eGFR >60 ml/min per 1.73 m^2 (n = 280, STEMI = 30)				
HScTnT >14.0 ng/L	93 [78−99]	87 [82−91]	45 [33−58]	99 [96−100]
In patients with eGFR ≤60 ml/min per 1.73 m^2 (n = 72, STEMI = 13)				
HScTnT >14.0 ng/L	100 [75−100]	54 [40−67]†	33 [20−50]	100 [87−100]
HScTnT >43.2 ng/L	92 [64−100]	88 [77−95]	63 [39−83]	98 [89−100]

NPV = negative predictive value; PPV = positive predictive value.
* p value <0.001 versus patients aged <70 years.
† p value <0.001 versus patients with eGFR >60 ml/min per 1.73 m^2.

a lower eGFR value, and more frequently had elevated HScTnT at admission and NSTEMI.

HScTnT concentrations were significantly higher in elderly patients: 20.9 (9.6−86.1) versus 3.9 (3.0−10.7) ng/L in the younger group (p <0.001), regardless of the final diagnosis. However, as for cTnI, HScTnT concentrations remained significantly higher in patients with AMI in comparison with patients without AMI, regardless of age category (Figure 1). HScTnT concentrations increased significantly whereas eGFR decreased: 3.0 ng/L (3.0−9.3) for eGFR ≥90 ml/min per 1.73 m^2, 5.9 ng/L (3.0−15.6) for eGFR from 60 to 89 ml/min per 1.73 m^2, and 16.9 ng/L (6.3−51.7) for eGFR ≤60 ml/min per 1.73 m^2; (p <0.001). As for cTnI, HScTnT concentrations remained significantly increased in patients with AMI compared with patients without AMI, regardless of the eGFR category (Figure 1).

The AUC for HScTnT to diagnose AMI did not differ in elderly patients (0.92 [0.85−0.97]) versus younger patients (0.93 [0.89−0.95], p = 0.960). Diagnostic performances of HScTnT are presented in Table 2. In elderly patients, the optimal threshold value for HScTnT based on ROC curve was 32.4 ng/L. Using that cutoff resulted in high sensitivity but somewhat lower specificity compared with that observed in younger patients. Bootstrapping analysis gave an optimal cutoff value at 53.5 ng/L [95% confidence interval: 34.3−109.7] that resulted in same sensitivity and specificity compared with younger patients. After exclusion of STEMI patients, the analysis was similar: AUC in elderly patients = 0.93 [0.84−0.97], p = 0.798 versus younger patients; optimal cutoffs are reported in Table 3. Sixty-two percent of elderly patients had HScTnT >14 ng/L at admission, 42% had a value >32.4 ng/L, and 32% were >53.5 ng/L. For comparison, 31% of elderly patients had cTnI values above the 10% CV value.

The AUC for HScTnT to diagnose AMI did not differ across estimated GFR categories: AUC = 0.96 [0.88−0.99] for eGFR ≤60 ml/min per 1.73 m^2; AUC = 0.91 [0.86−0.95] for eGFR from 60 to 89 ml/min per 1.73 m^2 (p = 0.467 vs eGFR ≤60 ml/min per 1.73 m^2); AUC = 0.95 [0.89−0.98] for eGFR ≥90 ml/min per 1.73 m^2 (p = 0.454 vs eGFR ≤60 ml/min per 1.73 m^2; p = 0.876 vs eGFR 60−89 ml/min per 1.73 m^2). On the basis of the ROC curve, the optimal threshold value for HScTnT was 35.8 ng/L in patients with low eGFR (Table 2). Bootstrap analysis

confirmed this threshold value (37.6 ng/L; [17.9−75.3]). A subanalysis in elderly patients with eGFR ≤60 ml/min per 1.73 m^2 (n = 40, AMI = 12) indicated a sensitivity of 100% [70%−100%], a specificity of 79% [59%−91%], a positive predictive value of 67% [42%−86%], and an negative predictive value of 100% [82%−100%], using the threshold value of 35.8 ng/L. After exclusion of STEMI patients, the analysis was similar. Optimal cutoffs are reported in Table 3.

Elderly patients without AMI tended to have more frequently elevated HScTnT (23%) than younger patients (12%, p = 0.06). In elderly patients without AMI and with elevated HScTnT values, the final diagnoses were as follows: UA (n = 2), cardiac causes (n = 6, including 2 stable angina, 2 pericarditis, and 2 acute heart failure), and noncardiac causes (n = 6). The rate of non-AMI patients with elevated HScTnT values was higher in eGFR ≤60 ml/min per 1.73 m^2 (37%) than in eGFR from 60 to 89 ml/min per 1.73 m^2 (13%) and eGFR ≥90 ml/min per 1.73 m^2 (10%; p = 0.007 for trend). For the lowest chronic kidney disease stages population with elevated HScTnT values (n = 26), the final diagnosis was UA (n = 4), cardiac cause (n = 6, including 2 stable angina, 2 pericarditis, 2 atrial fibrillation), and noncardiac causes (n = 16, including 1 syncope and 1 acute heart failure and 14 undocumented).

Patients with a positive HScTnT value were more frequently elderly (42% vs 22%, p <0.001), presented more frequently with diabetes (43% vs 24%, p = 0.005), more often had a history of heart failure (48% vs 25%, p = 0.024), had a low eGFR (37% vs 22%, p = 0.003), and had more frequently typical chest pain (33% vs 22%, p = 0.030). After adjustment, multivariate analysis indicated that age >70 years (odds ratio [OR] = 3.9 [2.2−7.1], p <0.001) was the strongest predictor of a positive HScTnT regardless of the final adjudicated diagnosis. A low eGFR (OR = 1.8 [1.0−3.0], p = 0.036), history of heart failure (OR = 2.8 [1.1−7.7], p = 0.038), and typical chest pain (OR = 1.8 [1.1−2.9], p = 0.018) were also predictors of a positive HScTnT at admission. After exclusion of patients with ST elevation, patients with a positive HScTnT value were more frequently elderly (37% vs 18%, p <0.001), had more frequently diabetes (25% vs 12%, p = 0.003), history of heart failure (13% vs 5%, p = 0.022), and a low eGFR (49% vs 29%, p = 0.001). Multivariate analysis indicated that age >70 years (OR = 3.5 [1.2−6.3], p <0.001), a low eGFR (OR = 2.0 [1.2−3.5], p = 0.010), and history of heart failure (OR = 3.2 [1.2−8.7], p = 0.023) were still independent predictors of a positive HScTnT.

Discussion

This study indicates that (1) HScTnT concentrations are slightly correlated with age and renal function and (2) using adapted thresholds for HScTnT results in high sensitivity and preserved specificity for the diagnosis of AMI or NSTEMI in elderly patients and in patients with renal failure.

Recent studies have demonstrated that HScTn assays increase the accuracy in the early diagnostic of AMI.[3−9] However, these studies included mostly middle-aged patients. In our study, 23% of patients were elderly, a finding consistent with previous studies.[10] Recently, Eggers et al found that elevated HScTnI levels were relatively common in elderly subjects and were associated with cardiovascular risk factors and/or impaired cardiac performance.[13] In contrast to previous work, our study highlights the significant correlation of age with HScTnT, whatever the adjudicated diagnosis.[13,23] The release of cTn from cardiomyocytes in healthy adult subjects may result from a "physiological remodelling" process.[10] Several histological changes, characterized by a loss of myocytes with subsequent hypertrophy of the remaining cells and the calcification of cardiac structures, can be found in most individuals as they are getting older.[24] HScTn values among patients without AMI were shown to be correlated to age.[10,16] Additional studies in apparently healthy populations demonstrated the relationship of HScTnT with age and renal function.[25−27] However, a recent study indicated that the influence of age on HScTnT was attenuated when allowance was made for other factors such as cardiorespiratory function.[28] If HScTn may increase in elderly patients as the consequence of some physiological processes as well as noncardiac diseases,[10] it might result in a possibly reduced specificity of the test. The recourse to an adapted cutoff may be useful, but the optimal value is not yet established. In a recent study, Reiter et al reported the metrics of different cutoffs. The optimal value was 54 ng/L in their elderly cohort, based on ROC curve analysis.[23] Our AUCs were similar, and both ROC and bootstrap cutoffs are also higher than that recommended initial reports for the whole population.[25] Interestingly, we reported that using a cutoff at 32.4 or 53.5 ng/L results in a specificity that is not significantly different from that observed in younger patients. Our study also indicates that sensitivity is not higher than that observed in younger patients. The small sample of elderly patients (n = 84) might explain the difference of cut-off values obtained from ROC and bootstrap analysis. Finally, we demonstrated that elderly was the most powerful predictor of a HScTnT above the 99th percentile at admission. In association to higher proportion of comorbidities, this is in line with the literature[26,27] and indicates that troponin elevations are not solely related to age but also to comorbidities and/or underlying cardiac disease.

Previous reports suggested that renal dysfunction influences cTn concentrations.[17] Geriatric cardiologists recommend that creatinine clearance should be calculated for every elderly patient to enable appropriate interpretation of cTn.[18] Correia et al previously demonstrated that moderate renal dysfunction was not associated with elevated conventional cTn in acute coronary syndromes.[29] Our study has shown that (1) the correlation of HScTnT with eGFR is weak but significant, (2) AUCs did not significantly vary according to eGFR categories, and (3) a higher optimal threshold value might be used (35.8 ng/L in patients with eGFR <60 ml/min per 1.73 m^2) for the diagnosis of AMI. Here again, we reported that using a higher cutoff for the diagnosis of AMI results in a specificity that is not significantly different from that observed in patients with normal eGFR values; the same observation is done when with a diagnosis of NSTEMI, using a slightly higher cut-off value (43.2 ng/L). To our knowledge, this is the first study that describes adapted cutoffs according to eGFR. Of note, our adapted-to-eGFR threshold value corresponds to mild increases that could not be detected using

conventional cTnT assay. Finally, a low eGFR was also an independent predictor of a HScTnT value >14 ng/L at admission, regardless of the final adjudicated diagnosis.

Our study should be interpreted within its limitation. First, this is a post hoc analysis of 2 prospective studies. As a consequence, we observed a somewhat limited prevalence of elderly patients. Second, we only evaluated the performances of a single measurement of HScTnT at admission. Recently published recommendations for the routine use of HScTn are based on a changing pattern of values for an optimal interpretation.[30] Third, our study was observational, and additional interventional studies seem warranted to quantify exactly the clinical benefit associated with the increase in early diagnostic accuracy in the subgroup of elderly patients. However, the goal of the study was to focus on the impact of age and renal function on the diagnostic accuracy of HScTnT, not to compare it with conventional cTn. Fourth, the optimal threshold values derived on ROC curves in different subgroup populations (elderly and with renal impairment) was not reinvestigated in a dedicated validation cohort. Therefore, our threshold values should be considered as preliminary report and remain to be confirmed in dedicated studies.

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Disclosures

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Approche multimarqueurs en médecine d'urgence

Résumé :

L'apport des biomarqueurs aux urgences est bien documenté. Depuis l'apparition de la myoglobine et de la troponine pour le diagnostic de syndrome coronaire aigu (SCA), de multiples marqueurs ont été développés pour l'aide au diagnostic de multiples pathologies aux urgences. Certains biomarqueurs sont même intégrés à la définition de syndromes ou pathologies comme le SCA avec la troponine, ou le sepsis sévère avec le lactate. Nous abordons dans ce travail l'approche multimarqueurs, qui consiste à combiner le dosage de plusieurs biomarqueurs pour améliorer les performances diagnostiques ou pronostiques. L'hypothèse de base de ce travail est que l'association d'un marqueur sensible, généraliste, avec un marqueur spécifique de pathologie ou de dysfonction d'organe, permettrait d'améliorer la prise en charge diagnostique ou la stratification du risque aux urgences.

On illustre cette approche dans trois cas particuliers : la prédiction du sepsis sévère, le diagnostic du syndrome coronaire aigu, et l'évaluation du risque après une crise convulsive. Plusieurs méthodes sont envisagées pour combiner plusieurs biomarqueurs, et on développera ici la détermination de la meilleure combinaison linéaire pour obtenir une discrimination optimale.

Mots clés : biomarqueurs - médecine d'urgence - courbe ROC – sepsis – syndrome coronaire aigu - convulsions

Multimarker approach in emergency medicine

Abstract:

The added value of biomarkers in the emergency settings is well reported, in various pathologies. Since the burst of myoglobin and troponine for the diagnosis of myocardial infarction (MI), various biomarkers have been developed and adopted for diagnostic purposes in different pathologies. Some of them are part of the very definition of specific syndrom or disease (MI with troponin, or severe sepsis with lactate). We present here the multimarker approach in the emergency department – a strategy that combines the results of several different biomarkers to enhance diagnostic or prognostic performances. We made the hypothesis that the association of a sensitive and generalist biomarker, with an organ or syndrome specific one, would result in better performances.

We illustrate here this strategy in three particular cases: the prediction of severe sepsis, the diagnosis of acute coronary syndrome, and the risk stratification after a convulsive seizure. Several methods are considered for the combination of biomarkers, and we will focus on the determination of the best linear combination.

Key words: biomarkers – emergency medicine – ROC curve – sepsis – acute coronary syndrome - seizure