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Determinação do valor do peptídeo natriurético tipo B na monitorização da resposta à terapêutica e da progressão dos estados de hipervolémia

Dissertação de candidatura ao grau de Doutor apresentada à
Faculdade de Medicina da Universidade do Porto

Porto, 2010

Artigo 48º, § 3º - “A Faculdade não responde pelas doutrinas expendidas na dissertação.”

(Regulamento da Faculdade de Medicina da Universidade do Porto.

Decreto-Lei n.º 19337 de 29 de Janeiro de 1931)

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Doutor Walter Friedrich Alfred Osswald

Ao abrigo do Art.º 8º do Decreto-Lei n.º 388/70 fazem parte desta dissertação as seguintes publicações:

I. Pimenta J, Almeida R, Araújo JP, Azevedo A, Friões F, Rocha-Gonçalves F, Ferreira A, Bettencourt P. Amino-terminal B-type natriuretic peptide, renal function, and prognosis in acute heart failure: a hospital cohort study. *J Card Fail* 2007;13:275-280.

II. Pimenta J, Sampaio F, Martins P, Carvalho B, Rocha-Gonçalves F, Ferreira A, Bettencourt P. Aminoterminal B-type natriuretic peptide (NT-proBNP) in end-stage renal failure patients on regular hemodialysis: does it have diagnostic and prognostic implications? *Nephron Clin Pract* 2009;111:182-188.

III. Pimenta J, Paulo C, Mascarenhas J, Gomes A, Azevedo A, Rocha-Gonçalves F, Bettencourt P. BNP at discharge in acute heart failure patients: Is it all about volemia? A study using impedance cardiography to assess fluid and hemodynamic status. *Int J Cardiol* 2009. doi:10.1016/j.ijcard.2009.08.001 [Epub ahead of print]

IV. Pimenta J, Paulo C, Gomes A, Silva S, Rocha-Gonçalves F, Bettencourt P. B-type natriuretic peptide is related to cardiac function and prognosis in hospitalized patients with decompensated cirrhosis. *Liver Int* 2010. doi:10.1111/j.1478-3231.2010.0226.x [Epub ahead of print]

A contribuição pessoal para a realização destes trabalhos foi a seguinte:

Contribuição importante na sua concepção, na recolha do material, obtenção e análise dos dados e redacção dos manuscritos.

Ao Francisco

À Inês e ao João Francisco

Aos meus Pais

Aos meus irmãos, Tiago, Adriana e Eduardo

À minha Família e aos meus Amigos

Ao Professor Doutor Paulo Bettencourt

Ao Professor Doutor António Ferreira

AGRADECIMENTOS

A investigação, particularmente na sua vertente clínica, constitui um desafio estimulante e um componente do exercício da Medicina. Foi nessa perspectiva que encarei a elaboração dos estudos que compõem a presente dissertação, que apresento à Faculdade de Medicina da Universidade do Porto. Esse conjunto de trabalhos não seria exequível sem o contributo abnegado e generoso de tantos que participaram, directa ou indirectamente, na sua realização e a quem expresso a minha gratidão. De entre todos, cumpre-me manifestar especial agradecimento:

Ao Professor Doutor Francisco Rocha Gonçalves pela confiança em mim depositada ao formular-me o convite para integrar a sua equipa docente e para desenvolver esta investigação. O seu estímulo e empenho constantes e a sua disponibilidade foram decisivos para a prossecução deste trabalho.

Ao Professor Doutor Paulo Bettencourt, Director do Serviço de Medicina Interna, Coordenador da Consulta de Insuficiência Cardíaca e Regente da disciplina de Semiótica Clínica, aos quais me orgulho de pertencer. As suas qualidades como Médico, Investigador e Docente são um modelo e um incentivo permanente à excelência. Agradeço, profundamente reconhecida, a colaboração na idealização e na avaliação crítica e minuciosa da investigação clínica efectuada, bem como a revisão pormenorizada e relevante de todos os trabalhos dela resultantes e desta dissertação. A estreita colaboração que desenvolvemos ao longo deste período consolidou ainda mais a amizade e o reconhecimento que lhe devoto.

Ao Professor Doutor António Ferreira, pelo contributo fundamental para a minha formação clínica, científica e académica e pelo exemplo de perseverança, determinação e

rigor científico. Agradeço a confiança que demonstrou ao integrar-me, numa fase precoce da minha formação, na equipa da Consulta de Insuficiência Cardíaca, e que moldou, de forma decisiva, o gosto e interesse pela investigação clínica. A ele me liga, também, uma profunda consideração e amizade.

À Professora Doutora Ana Azevedo, pelo empenho e disponibilidade sempre demonstradas no acompanhamento da análise estatística e revisão crítica dos estudos realizados. O seu conhecimento e rigor científico e a sua frontalidade contribuíram de forma relevante para a elaboração desta dissertação e para a minha formação na investigação clínica.

À Dr.ª Cristiana Paulo, à Dr.ª Joana Mascarenhas, ao Dr. André Gomes pelo esforço, dedicação e entusiasmo que devotaram ao trabalho de campo que conduziu a esta dissertação. A sua ajuda preciosa e o seu apoio incondicional foram indispensáveis a este projecto e reforçaram a amizade que nos une.

À Dr.ª Berta Carvalho e à Dr.ª Patrícia Martins, agradeço a prestimosa colaboração na avaliação dos doentes com insuficiência renal terminal e disponibilidade para a discussão crítica dos seus resultados.

Aos colegas do Serviço de Medicina Interna, particularmente aos que me acompanharam mais directamente no quotidiano da prática clínica na Consulta de Insuficiência Cardíaca – Prof. Doutor José Paulo Araújo, Dr. Fernando Friões, Dr.ª Margarida Alvélos e Dr. Sérgio Silva – e no Internamento – Dr.ª Anabela Gonçalves e Dr.ª Fátima Coelho – pela compreensão, a ajuda e o apoio amigo que sempre me manifestaram.

Ao corpo de enfermagem do Serviço de Medicina Interna, expresso a minha gratidão pela colaboração profissional e diligente na colheita de amostras para os vários estudos desta tese.

Aos doentes e controlos que de forma generosa aceitaram participar neste projecto quero também manifestar o meu reconhecimento.

Ao Francisco, por partilhar a vida comigo, comungando as alegrias e sendo um esteio nas adversidades.

À Inês e ao João Francisco, fonte diária de energia, alegria, entusiasmo e amor incondicional.

Aos meus Pais, que me formaram e sempre me incentivaram a cultivar o conhecimento e um espírito crítico e aberto. O seu exemplo, e o dos meus Avós, permanece como inspiração.

Aos meus irmãos, e restante família, pela convivência, encorajamento e compreensão.

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I. Introdução

1.1. OS PEPTÍDEOS NATRIURÉTICOS

A manutenção da homeostasia interna dos seres vivos condiciona a sobrevivência. Deste modo, os mecanismos biológicos destinados à preservação, em limites relativamente estreitos, da composição interna dos seres vivos têm sido apurados ao longo da evolução filogenética, nomeadamente com a transição do meio aquático para o meio terrestre.

O sistema dos peptídeos natriuréticos (PN) parece ter surgido neste contexto, como um mecanismo regulador da excreção de sódio e água, permitindo fazer face à desidratação e à gravidade implicados no processo adaptativo à vida terrestre. É constituído por um grupo de peptídeos filogenética, estrutural e funcionalmente relacionados, cuja principal função é a regulação da homeostasia hidroelectrolítica.

1.1.1. Biologia dos peptídeos natriuréticos

Em 1968, de Bold e colaboradores iniciaram o estudo da composição química e propriedades biológicas de grânulos auriculares específicos, que culminou na descrição, em 1981, dos efeitos diurético e natriurético da infusão de homogeneizados de extractos auriculares cardíacos no rato[1]. A constatação de que os extractos auriculares continham um factor natriurético conduziu à investigação que permitiu identificar o "atrial natriuretic peptide" – ANP[2].

Posteriormente foram sendo descritos novos compostos estruturalmente similares ao ANP mas geneticamente distintos, com várias acções na homeostasia cardiovascular, renal e endócrina, e que constituem, juntamente com o ANP, a família dos PN: o PN tipo B (BNP), o PN tipo C (CNP), o PN tipo D (DNP) e a urodilatina, um peptídeo de expressão exclusivamente renal que resulta de processamento alternativo do gene do ANP no rim[3,4,5,6].

Cada um destes peptídeos tem uma distribuição tecidular e regulação específicas: o ANP e o BNP são primariamente expressos nos cardiomiócitos, o CNP no cérebro e endotélio e o DNP foi identificado no miocárdio e plasma humanos. No entanto, todos partilham uma estrutura comum: um anel central com 17 aminoácidos (aa), com uma sequência altamente conservada, formado por uma ligação dissulfídrica entre dois resíduos de cisteína, essencial à actividade biológica, e extremidades carboxilo- (C) (ausente no CNP) e amino- (N) terminais variáveis. Todos os PN são sintetizados como precursores (pró-hormonas), sofrendo posteriormente modificações pós-translacionais que dão origem à forma activa destas hormonas.

O ANP, isolado e sequenciado em 1983, é, na forma circulante activa C-terminal, um peptídeo de 28 aa (ANP99-126), que deriva do próANP (ANP1-126), a sua forma de armazenamento nos grânulos auriculares específicos dos cardiócitos. Este precursor resulta, por sua vez, do processamento de um polipeptídeo de 151 aa (pré-próANP). A clivagem da pró-hormona em ANP activo (fragmento C-terminal) e no fragmento N-terminal de 98 aa parece ocorrer durante a secreção e ser, pelo menos em parte, resultante da acção de uma protease da serina cardíaca (corina)[7,8]. O ANP é sintetizado maioritariamente nas aurículas cardíacas e, em menor quantidade, nos ventrículos e cérebro. Em situações fisiológicas, é o PN predominante em circulação, reflexo da sua maior abundância nos grânulos, por comparação com o BNP. O gene do ANP localiza-se no cromossoma 1p36.2 humano e compreende 3 exões e 2 intrões[8]. Em animais de experiência, a destruição específica do gene do ANP (ratos "knockout" para o gene do ANP) resultou no desenvolvimento de hipertensão sensível ao sal[9].

Embora o BNP tenha sido identificado inicialmente no cérebro porcino e se encontre também no cérebro e aurículas humanos, é nos ventrículos cardíacos (e principalmente no esquerdo) onde é sintetizado em maior quantidade, inicialmente sob a forma de um precursor, pré-próBNP1-134, que sofre remoção de um peptídeo de sinal de 26 aa, resultando na molécula de próBNP1-108. O processamento do próBNP, que provavelmente ocorre na altura da secreção[10] por acção da corina (exclusivamente presente no miocárdio) ou da furina (presente em vários tecidos e no soro), origina o BNP activo (32aa) e um fragmento N-terminal biologicamente inactivo (NT-próBNP1-76)[11]. Estes peptídeos são

libertados em circulação, em concentrações muito baixas nos indivíduos saudáveis (da ordem dos 5 picomolar no sangue venoso), mas em quantidades apreciáveis (que podem ascender até mais de 1000 vezes) em situações patológicas específicas, tornando-se, então, o BNP no PN predominante a nível plasmático e tecidular. Em circunstâncias fisiológicas, a maior quantidade do BNP circulante tem origem auricular, enquanto nas patológicas a sua produção é maioritariamente ventricular. O BNP é, por isso, muitas vezes descrito como uma hormona ventricular[12]. O BNP1-32 em circulação é provavelmente degradado de forma rápida em fragmentos, que predominam em relação ao BNP1-32, mas que são medidos pelos testes de BNP comercializados. Um dos fragmentos em circulação é o BNP3-32, que resulta da clivagem do BNP1-32 pela dipeptidil peptidase IV[13]. Curiosamente, foi descrita uma total ausência da forma madura de BNP1-32 em doentes com insuficiência cardíaca, que apresentavam níveis elevados de BNP nos testes disponíveis comercialmente[14]. O próBNP1-108 também existe em circulação nos doentes com insuficiência cardíaca[15]. O gene do BNP é composto por 2 exões e 1 intrão e localiza-se no cromossoma 1p36, aproximadamente 8 kilobases a montante do gene do ANP[16]. Os ratos "knockout" para o gene do BNP desenvolvem lesões fibróticas multifocais nos ventrículos cardíacos, na ausência de hipertensão sistémica ou de hipertrofia ventricular[17,18].

Foram isolados 2 peptídeos resultantes do precursor próCNP (126 aa): um de 22 aa predominante no sistema nervoso central, hipófise anterior, rim, células endoteliais vasculares, macrófagos e plasma e um de 53 aa, menos potente e de menor expressão, que é uma extensão amino-terminal do CNP-22[19]. Ao contrário do ANP e BNP, o CNP não é produzido pelo coração, tendo origem primariamente vascular. A concentração plasmática de CNP é muito baixa e actualmente são-lhe atribuídas acções vasodilatadoras e antiproliferativas essencialmente a nível autócrino/parácrino[20]. O gene do CNP encontra-se no cromossoma 2 humano e contém 2 exões separados por um intrão[21].

O DNP, um peptídeo de 38 aa, foi isolado mais recentemente do veneno da cobra *Dendroaspis angusticeps* e identificado no miocárdio e plasma humanos, encontrando-se aumentado nos doentes com insuficiência cardíaca[22]. Contudo, não foi ainda identificado o gene humano do DNP. As suas acções são semelhantes às do PN cardíacos, ANP e BNP, tendo um potente efeito diurético e natriurético[23].

A urodilatina é um PN exclusivamente renal, inexistente na circulação sistémica, isolado na urina humana em 1988[6]. É processado no rim, a partir do mesmo percursor do ANP mas com uma extensão de 4 aa na região N-terminal (ANP95-126). A sua síntese e secreção intra-renal são a base de um sistema parácrino de regulação da reabsorção de sódio e água ao nível do tubo colector, já que este peptídeo é sintetizado no túbulo distal e actua nos receptores dos PN tipo A (NPR-A) na face luminal das células do tubo colector. O ANP99-126 e a urodilatina partilham características bioquímicas e funções biológicas, mas a urodilatina poderá estar mais envolvida na regulação do volume de fluído corporal e excreção de água e electrólitos, enquanto o ANP circulante será responsável pela regulação da tensão arterial[24,25].

Os PN exercem os seus efeitos através da interacção com receptores de superfície de alta afinidade presentes nas células-alvo, situadas em múltiplos locais do organismo (incluindo rim, músculo liso vascular, supra-renal, intestino, coração, cérebro, osso, pulmão, gónadas). Foram descritos três tipos de receptores dos PN (NPR): A, B e C. Existe ainda um receptor tipo D, que ainda só foi isolado na enguia e cuja função é ainda desconhecida.

Os tipo A e B são proteínas transmembranares, estão ligados a uma cascata de sinalização intracelular dependente do monofosfato de guanidina cíclico (GMPc), são estruturalmente similares e medeiam muitos dos efeitos cardiovasculares e renais dos PN. O NPR-A liga-se ao ANP e ao BNP – com uma afinidade cerca de 10 vezes maior para o primeiro – sendo o receptor que medeia a maioria dos efeitos biológicos destes dois peptídeos (não foi ainda descrito um receptor com afinidade preferencial para o BNP). O NPR-A é expresso abundantemente nos vasos, rins e glândulas supra-renais[26-29]. A ligação dos PN ao domínio de conexão ao ligando do NPR-A resulta na produção intracelular do segundo mensageiro GMPc, que por sua vez vai condicionar as respostas resultantes da estimulação do NPR-A: natriurese, inibição da renina e aldosterona, vasodilatação, efeitos lusitrópicos, anti-fibróticos e anti-hipertróficos. O CNP é o ligando preferencial do NPR-B, que é expresso predominantemente no cérebro, incluindo hipófise, e que poderá ter um papel na regulação neuroendócrina. O NPR-B tem também expressão periférica ao nível do coração, vasos, rins e tracto gastrointestinal. Há um

predomínio do NPR-B nas veias e a sua activação conduz a vasorelaxamento e consequentemente a um efeito hipotensor; parece não estar envolvido na resposta natriurética e diurética induzida pelos PN.

Em ambos os receptores, a interacção ligando-receptor resulta na activação de um domínio "cínase-like" intracelular, condicionando a activação da guanilcíclase e levando, assim, a um aumento do GMPc intracelular. O GMPc exerce os seus efeitos indirectamente (através da proteína cínase G GMPc-dependente) ou directamente (por acção nos efectores – *p.e.* canais de sódio amiloride-sensíveis no rim).

O terceiro receptor dos PN, NPR-C, é essencialmente um receptor de depuração dos PN, embora se admita que também possa ter funções de sinalização independentes. É um homodímero transmembranar, homólogo do NPR-A e -B no seu domínio extracelular, mas com um domínio citoplasmático curto, sem actividade guanilcíclase mas contendo uma sequência activadora das proteínas G. Liga-se aos PN segundo a ordem de afinidade ANP>CNP>BNP[30]. A menor afinidade do NPR-C para o BNP contribui para uma maior semi-vida plasmática deste PN comparado com o ANP no Homem. A interacção PN/NPR-C condiciona a internalização do complexo ligando-receptor, sendo depois os PN degradados enzimaticamente e os receptores reciclados e reexpressos à superfície celular[29]. O NPR-C é expresso em abundância em múltiplos tecidos como a maioria das glândulas endócrinas major, pulmões, rins e parede vascular[29]. O NPR-C poderá também estar envolvido nos efeitos antiproliferativos do BNP e CNP.

Os PN em circulação são também inactivados por clivagem pela endopeptidase neutra 24.11 (NEP), uma metalopeptidase de zinco, presente à superfície de células endoteliais, musculares lisas, cardiócitos, células do epitélio renal (tubular e glomerular) e fibroblastos[31]. O ANP parece ser muito mais susceptível à hidrólise pela NEP do que o BNP[32]. Fruto dessa menor susceptibilidade para a degradação enzimática e também para a ligação ao receptor de depuração, o BNP tem uma semi-vida plasmática consideravelmente maior que o ANP (cerca de 20 minutos *versus* 3 minutos), facilitando a sua quantificação analítica. A contribuição relativa da NEP e do NPR-C para a eliminação do BNP é provavelmente semelhante[33] e o BNP é também eliminado pelo rim[34].

Os fragmentos N-terminais (NT) resultantes do processamento das pró-hormonas dos PN, como o NT-próBNP, não têm um receptor de depuração específico, como o NPR-C, nem sofrem degradação pela NEP. Por esse motivo, têm uma semi-vida maior (especialmente o NT-ANP) e, consequentemente, uma concentração plasmática mais elevada e estável, menos sujeita à influência de pequenos pulsos de secreção. Estas características levaram a uma progressiva utilização do doseamento dos fragmentos NT dos PN, em alternativa à determinação da fracção activa do ANP e BNP, cuja semi-vida é de cerca de alguns minutos[35]. O NT-próBNP é filtrado da circulação pelos rins, proporcionalmente ao BNP[34,36].

Assim, e apesar da maioria dos efeitos do BNP ser também mediada pelos outros PN cardíacos, o BNP e o NT-próBNP são os biomarcadores do sistema dos PN mais estudados e utilizados na prática clínica, fruto da maior estabilidade das suas concentrações plasmáticas no Homem. Isto condicionou o desenvolvimento de vários testes de quantificação destes PN pelas empresas de diagnóstico biomédico.

1.1.2. Estímulos que induzem a síntese dos PN e sua regulação

A expressão génica e secreção dos PN cardíacos (ANP e BNP) pode ocorrer em situações distintas. Por um lado, resulta da normal expressão fenotípica do "coração endócrino", as aurículas, na vida adulta. Por outro, acontece durante o desenvolvimento ontogénico, no ventrículo fetal, e em situações patológicas determinadas, *p.e.* nos ventrículos hipertrofiados. Vários trabalhos sugerem que a regulação da síntese do ANP e BNP é diferente nas aurículas e nos ventrículos[37].

Os genes do ANP e do BNP são expressos de forma altamente específica nos miócitos cardíacos, mas não nos esqueléticos, sendo o estiramento da parede miocárdica o principal determinante da sua activação[37]. *In vivo*, observa-se indução da expressão génica do ANP um dia após início de sobrecarga cardíaca[38], enquanto o estiramento da parede provoca activação da expressão do gene do BNP em cerca de uma hora nas aurículas e ventrículo esquerdo[39,40]. A sobrecarga cardíaca crónica também causa um aumento dos níveis ventriculares do ácido ribonucleico mensageiro (RNAm) do BNP[41]. Em consonância com estes achados, encontram-se níveis elevados dos PN cardíacos

em situações de sobrecarga cardíaca de volume ou de pressão, as quais parecem, assim, constituir estímulos fisiológicos para a expressão génica e secreção de BNP[42].

Embora os estímulos mais importantes na indução da translacção do gene do BNP sejam o estiramento e a isquemia/hipóxia dos cardiomiócitos, outros estímulos endócrinos, parácrinos e autócrinos condicionam também a síntese de BNP, como a endotelina-1, angiotensina II, interleucina-1ß e os agonistas adrenérgicos[43]. De Bold e colaboradores descrevem a sobreexpressão de BNP, mas não de ANP, durante os episódios de rejeição aguda de aloenxertos cardíacos, colocando a possibilidade de esse fenómeno resultar da acção de citocinas[37,44].

O aumento da expressão génica do BNP em resposta à sobrecarga mecânica poderá resultar de mecanismos transcripcionais, pós-transcripcionais ou de ambos[42].

A transcrição génica do ANP e do BNP, tal como a de outros genes cardio-específicos, parece ser caracterizada por múltiplas interacções combinatórias de factores de transcrição, entre os quais a família GATA cardíaca. As contribuições individuais ou combinatórias dos GATA-4, -5 e -6 para o desenvolvimento e/ou regulação da expressão génica dos PN e as cascatas de sinalização que modulam essas interacções permanecem ainda por clarificar, mas o seu esclarecimento poderá tornar os factores GATA em potenciais novos alvos farmacológicos[42].

O RNAm do BNP, ao contrário do RNAm do ANP, apresenta quatro sequências repetitivas “AUUUAA” na região 3’-UTR[45]. Estas sequências ricas em AU encontram-se muitas vezes nas citocinas e protooncogenes e são responsáveis por conferir instabilidade ao RNAm, podendo estar envolvidas na rápida degradação do RNAm dependente da tradução[46]. Este facto sugere que o RNAm do BNP apresenta um “turnover” elevado e que o BNP é sintetizado em pulsos, destinado a respostas rápidas a variações fisiológicas, funcionando como uma hormona cardíaca “de emergência”[47].

O BNP e o ANP sofrem transformação pós-translacional, tal como outras hormonas polipeptídicas, por pregueamento e processamento proteolítico durante o seu trajecto a partir do retículo endoplasmático, através do complexo de Golgi, para os grânulos secretórios, onde ANP e BNP coexistem, embora este último em menor quantidade. Estes grânulos constituem um local de armazenamento de onde as hormonas podem

ser libertadas em resposta a estímulos apropriados e independentemente da síntese hormonal[37]. Assim, a regulação da libertação de ANP ocorre principalmente a nível da secreção hormonal, sendo necessária uma diminuição crítica do ANP armazenado para que ocorra activação transcripcional. Por outro lado, a regulação da síntese e excreção do BNP ocorre, como já vimos, principalmente a nível da expressão génica.

1.1.3. Efeitos biológicos dos PN

Os PN regulam vários parâmetros fisiológicos, como a pressão arterial e o volume intravascular, desempenhando um papel fundamental na resposta à hipertensão e à expansão de volume. Estes efeitos resultam de acções coordenadas em vários locais, nomeadamente cérebro, vasos, rins e supra-renais.

1. Acções cardiovasculares

O ANP e o BNP têm acções muito similares a nível cardiovascular. O seu efeito hipotensor resulta, em parte, da redução da pré-carga por aumento da permeabilidade capilar (e consequente redução do fluído intravascular), aumento da capacitância venosa e promoção da natriurese (*vide infra*), levando à redução do volume do líquido extracelular. Os PN induzem ainda redução das resistências vasculares pulmonares e sistémicas (pós-carga), através do relaxamento do músculo liso vascular, e das pressões telediastólicas auriculares e ventriculares. Estes efeitos condicionam um aumento do débito cardíaco e da função diastólica. O BNP tem propriedades lusitrópicas directas[48-52].

O efeito supressor do tónus simpático nos vasos periféricos, resultante da inibição da activação dos barorreceptores e da supressão da libertação de catecolaminas nos terminais nervosos periféricos e, principalmente, no sistema nervoso central, contribui também para o mecanismo anti-hipertensor dos PN. Além disso, o ANP reduz o limiar de activação dos aferentes vagais, suprimindo a resposta reflexa à redução da pré-carga (taquicardia e vasoconstrição) e garantindo uma diminuição sustentada da pressão arterial. O ANP e o BNP bloqueiam a actividade simpática cardíaca, mesmo quando há diminuição das pressões de enchimento cardíacas[48-52].

Os PN têm ainda um efeito anti-mitogénico e anti-proliferativo a nível cardiovascular, modulando o crescimento e proliferação celular e a hipertrofia cardiomiocitária (*vide supra*). O ANP e o CNP têm efeito anti-mitogénico sobre as células vasculares[53,54].

2. Acções renais

Os PN promovem uma resposta diurética e natriurética por mecanismos hemodinâmicos e por acções directas ao nível dos túbulos renais[6,48,51,52].

A dilatação das arteríolas aferentes e a constrição das arteríolas eferentes condicionada pelos PN causam elevação da pressão ao nível dos capilares glomerulares, resultando no aumento da filtração glomerular[55]. Por outro lado, os PN induzem o relaxamento das células mesangiais (além de inibirem a sua proliferação e migração, como já foi referido), levando a um aumento da área efectiva de filtração[56]. Além disso, o ANP poderá alterar a distribução do fluxo sanguíneo intra-renal, alterando a hemodinâmica medular e promovendo a natriurese[57]. Assim, os PN condicionam preservação (ou mesmo melhoria) da taxa de filtração glomerular, apesar da diminuição da pressão arterial média que provocam.

Além das acções hemodinâmicas, os PN têm efeitos natriuréticos por acção directa nos túbulos, independente do aumento da filtração glomerular. No túbulo proximal inibem o transporte de sódio e água estimulado pela angiotensina II[58]. Por outro lado, antagonizam a acção da vasopressina sobre o transporte de água nos tubos colectores corticais[59] e bloqueiam directamente a absorção de sódio nos colectores medulares, parecendo ser este o principal efeito a nível tubular[60,61]. Adicionalmente a esta acção antagónica sobre a vasopressina e angiotensina II a nível tubular, os PN têm também acção inibitória sobre o eixo renina-angiotensina-aldosterona[50], inibindo a libertação de renina e aldosterona, embora por mecanismos ainda incertos (a redução da aldosterona poderá resultar da acção directa dos PN na SR ou ser secundária à inibição da renina)[6,62,63].

3. Acções na supra-renal

Os PN inibem a síntese de aldosterona na supra-renal[63,64]. Recentemente foi colocada a hipótese de existir síntese de aldosterona a nível cardíaco e do BNP poder ter acção inibitória dessa síntese[65].

4. Acções no sistema nervoso central

Todos os PN são sintetizados no cérebro e os seus efeitos a este nível reforçam as suas acções periféricas[48]. Ocorre inibição do apetite para a ingestão de sódio e água[66,67] e está descrita a inibição da secreção de vasopressina e hormona adrenocorticotrófica[68]. A nível do tronco cerebral diminuem o tónus simpático[48].

5. Outras acções

Estudos mais recentes permitiram atribuir aos PN um espectro inesperadamente vasto de funções, nomeadamente a nível do desenvolvimento e metabolismo ósseo[69,70], da lipólise[71,72], da função imunitária dos macrófagos[73,74] e da erecção peniana[75].

1.1.4. Patofisiologia dos peptídeos natriuréticos

Os PN, principalmente o ANP e o BNP, constituem uma resposta de defesa às situações de excesso de retenção de sódio e água, associadas a sobrecarga de volume e pressão cardíacas. Seria, portanto, de prever o aumento plasmático desses peptídeos nas situações em cuja patofisiologia impera a hipervolémia e a distensão cardíaca. Parece haver uma relação mais estreita entre a pressão auricular esquerda e o ANP e entre os índices de volume e pressão ventriculares esquerdos e o BNP, embora haja sobreposição dos locais de libertação destes PN[51].

Existe um vasto conjunto trabalhos que suporta o papel fundamental do BNP na patofisiologia de várias doenças, permitindo estabelecer uma associação entre níveis plasmáticos elevados desse peptídeo e a presença e progressão de várias entidades clínicas.

Com efeito, estão descritas elevações do BNP sérico em patologias caracterizadas por expansão de volume (insuficiência cardíaca, insuficiência renal, cirrose hepática com ascite e hiperaldosteronismo primário) ou por estimulação da produção do peptídeo

(hipertrofia ou sobrecarga de pressão ventricular esquerda, hipertensão pulmonar, hipóxia, produção ectópica por tumor, patologia tiroideia, excesso de glicocorticóides circulantes)[76-79]. Além destas, outras circunstâncias podem estar associadas a aumento dos níveis de BNP: redução da depuração renal (insuficiência renal)[76], fármacos (inibidores da endopeptídase neutra e da vasopeptídase e bloqueadores beta)[76, 80].

A obesidade associa-se a níveis mais baixos de BNP/NT-próBNP[81-83], apesar dos doentes obesos terem pressões de enchimento ventriculares esquerdas superiores. Essa associação poderá implicar a utilização de um limiar diagnóstico inferior para os níveis de BNP nos obesos, mas aparentemente tal não sucederá no que respeita aos níveis de NT-próBNP[82,83].

Tem sido consistentemente descrita uma correlação inversa entre os valores de hemoglobina e os níveis de BNP e NT-próBNP, em indivíduos com e sem insuficiência cardíaca[84,85]. Embora a hemodiluição tenha sido apontada como o elo causal, poderão estar também implicados outros factores associados à anemia que podem estimular directamente a síntese de BNP, como a hipóxia miocárdica e a activação neurohumoral[85].

Na população geral estão documentados níveis superiores em idosos, recém-nascidos, mulheres e durante o terceiro trimestre da gravidez e puerpério[86-89]. O ANP apresenta variações rápidas e consideráveis em indivíduos saudáveis (com a postura, exercício, sobrecarga salina), ao contrário do que sucede com o BNP, reflexo provável da diferente regulação da secreção destes dois PN[76]. Parece existir pouca variação circadiana nos níveis de BNP[87,90].

À excepção da insuficiência renal, todas as outras possíveis influências nas concentrações de PN parecem ter pouca relevância na prática clínica, embora devam ser consideradas na interpretação de níveis de PN ligeiramente aumentados[52].

Recentemente foi proposto existir um componente genético substancial (de cerca de 40%) na variação dos níveis dos PN, em associação a características clínicas e ecocardiográficas[91]. Esta influência da hereditariedade nos níveis dos PN permite especular que uma predisposição genética para níveis baixos de PN poderá corresponder a uma maior susceptibilidade para desenvolver doenças associadas a deficiente excreção salina.

Em determinadas situações patológicas, o interesse da determinação dos níveis plasmáticos dos PN, nomeadamente ANP, BNP e seus fragmentos N-terminais está já bem esclarecido. De um modo geral, o BNP e NT-próBNP têm uma acuidade diagnóstica e prognóstica superior ao ANP e NT-ANP[52,92-97].

Insuficiência cardíaca

Os níveis plasmáticos de BNP encontram-se aumentados na insuficiência cardíaca[76,98-100]. Essa elevação correlaciona-se com a gravidade da apresentação clínica (em termos de classe funcional segundo a classificação da "New York Heart Association"), com parâmetros hemodinâmicos (como a pressão de oclusão da artéria pulmonar e a pressão telediastólica ventricular esquerda), com o grau de disfunção ventricular e com o prognóstico[51,76].

O interesse do BNP no diagnóstico de insuficiência cardíaca é considerável em populações de risco, como, por exemplo, na abordagem inicial de doentes com sintomas sugestivos de insuficiência cardíaca, quer em cuidados primários[93], quer no serviço de urgência[92,101-104], principalmente pelo seu alto valor preditivo negativo. Este bom desempenho do BNP no diagnóstico de insuficiência cardíaca levou à sua inclusão no algoritmo de diagnóstico dessa patologia proposto pela Sociedade Europeia de Cardiologia[105].

As concentrações plasmáticas de BNP têm também importante valor prognóstico na insuficiência cardíaca, correlacionando-se com mortalidade global e cardiovascular e com morbilidade (readmissão por insuficiência cardíaca e eventos após ida ao serviço de urgência por insuficiência cardíaca)[51,106-114]. A sua determinação poderá, assim, constituir uma importante mais-valia na estratificação de risco desta patologia.

Disfunção ventricular esquerda assintomática

Apesar desta situação cursar com aumento dos níveis de BNP, a sua concentração plasmática é menor do que na insuficiência cardíaca[76]. Este facto condiciona uma menor utilidade do BNP no diagnóstico desta entidade, principalmente no rastreio da população geral (onde a probabilidade pré-teste é baixa). Com efeito, vários estudos apontam para uma utilidade diagnóstica modesta do doseamento do BNP neste con-

texto, tendo sido proposto o seu uso essencialmente como teste de exclusão (*i.e.* tendo em vista evitar a realização de estudos subsequentes de avaliação de função cardíaca em indivíduos com valores baixos de BNP)[76,115-120]. Em populações seleccionadas com elevado risco de disfunção cardíaca (*p.e.* pós-enfarte do miocárdio, hipertensos, diabéticos, insuficientes renais terminais, indivíduos sob quimioterapia com antraciclinas) o papel do BNP no rastreio de disfunção ventricular assintomática, apesar de ainda não totalmente estabelecido, parece ser muito mais promissor[51,76]. Vários estudos apoiam a associação do BNP com o prognóstico pós-enfarte do miocárdio (nomeadamente com mortalidade cardiovascular), levantando a hipótese do doseamento do BNP facilitar a estratificação de risco nesta circunstância[52,76,95,120-123]. Foi também demonstrada a associação de níveis elevados de BNP com mortalidade global em indivíduos da população geral, com e sem insuficiência cardíaca, conferindo-lhe importante valor prognóstico[124,125].

Disfunção diastólica

Há um aumento consistente dos níveis de BNP nas patologias associadas a disfunção cardíaca diastólica, nomeadamente na estenose aórtica, cardiomiopatia hipertrófica e restritiva[126-129]. Essa elevação do BNP é de proporção inferior à verificada nos doentes com disfunção sistólica. Os indivíduos com comprometimento de ambas as funções sistólica e diastólica apresentam as concentrações séricas mais elevadas de BNP[130]. Nos doentes com função sistólica preservada, os níveis de BNP correlacionam-se com os índices Doppler de disfunção diastólica, *i.e.*, estão aumentados no padrão de alterações do relaxamento ventricular mas são mais elevadas no padrão restritivo[131,132]. A acuidade do BNP para detectar disfunção diastólica isolada aproxima-se do desempenho deste PN no diagnóstico de insuficiência cardíaca com disfunção sistólica[132], podendo constituir um meio simples de diagnosticar uma patologia cuja caracterização permanece ainda controversa, dada a ausência de critérios de diagnóstico adequados.

Lesão pulmonar/Disfunção ventricular direita

O papel do BNP nas patologias que envolvem disfunção ventricular direita e lesão pulmonar, apesar de menos estudado, parece ser de interesse considerável. Foi descrita elevação dos níveis de BNP em doentes com hipertensão pulmonar primária, embolia pulmonar, cardiopatia congénita, *cor pulmonale* e displasia arritmogénica do ventrículo direito[103,133-136]. Essa elevação é proporcional ao grau de disfunção ventricular direita nas situações de hipertensão pulmonar (primária, tromboembólica e cardiopatias congénitas) e correlaciona-se com parâmetros hemodinâmicos[133]. Nas situações de doença respiratória crónica foi encontrada uma correlação inversa entre os níveis de BNP e o grau de hipoxemia, explicável pelo possível efeito compensatório desse peptídeo na redução da vasoconstrição desencadeada pela hipóxia[133]. Os doentes com *cor pulmonale* têm elevação dos níveis de BNP, presumivelmente com origem no aumento da sua síntese no ventrículo direito. A sobrecarga de pressão do ventrículo direito corresponde a níveis superiores de BNP relativamente à sobrecarga de volume[133]. O aumento do BNP nas patologias cardíacas direitas parece ser proporcionalmente menor do que o resultante de disfunção ventricular esquerda.

Foi estabelecida a associação entre BNP e prognóstico na hipertensão pulmonar primária. Verifica-se um aumento substancial da mortalidade nos doentes com esta patologia que apresentam elevação dos níveis de BNP, especialmente se essa elevação persiste durante um curso de terapêutica vasodilatadora ou se os níveis aumentam ao longo do seguimento[137]. Na doença respiratória crónica o BNP é, também, uma variável independente de prognóstico, associada com a mortalidade[138].

Monitorização da terapêutica da insuficiência cardíaca

Nesta patologia a terapêutica baseia-se na melhoria dos sinais e sintomas congestivos e na titulação de fármacos até serem atingidas as doses que se demonstraram eficazes nos grandes ensaios clínicos. No entanto, seria desejável o estabelecimento de um marcador objectivo da eficácia terapêutica. Desde 1999 têm surgido alguns estudos que comparam a terapêutica empírica da insuficiência cardíaca, baseada na clínica, com a terapêutica dirigida à redução dos níveis de BNP e NT-próBNP[139-144]. Estes tra-

balhos demonstraram benefícios na titulação dos IECA e bloqueadores beta segundo os níveis dos PN, apontando para a sua determinação como um método para ajuste terapêutico na insuficiência cardíaca, especialmente nos doentes com menos de 75 anos[143,144]. A nível individual, o doseamento de BNP ou NT-próBNP pode ser útil como indicador prognóstico, identificando os doentes com valores elevados destes PN apesar de terapêutica optimizada como um grupo de alto-risco, que beneficiará de tratamento intensivo[52].

Utilização na terapêutica da insuficiência cardíaca

Os PN têm propriedades únicas, que os distinguem dos fármacos convencionais usados no tratamento da insuficiência cardíaca, já que, apesar de diminuirem a tensão arterial, não causam aumento da frequência cardíaca ou activação do sistema renina-angiotensina-aldosterona. Os níveis de PN encontram-se, como já foi referido, aumentados na insuficiência cardíaca, por vezes com uma magnitude de cerca de 1000 vezes superior aos dos níveis fisiológicos. Este aumento resulta da resposta compensatória do coração endócrino, mas traduz, também, uma certa dessensibilização do organismo aos efeitos dos PN. A explicação para este fenómeno parece ser multifactorial e englobar a sub-regulação dos NPR-A, o aumento da expressão dos NPR-C ou da actividade da NEP e ainda a existência de um estado de deficiência relativa, comparável à resistência à insulina observada nos diabéticos tipo 2[145]. A administração de PN exógenos conduz a efeitos similiares ao dos PN endógenos (vasodilatação, natriurese e diurese), que ocorrem mesmo na presença de níveis aumentados destes últimos. Este facto apoia a hipótese de existir um estado de deficiência relativa de PN na insuficiência cardíaca[145].

A utilização da infusão endovenosa de BNP humano sintético (nesiritide) em doentes com insuficiência cardíaca provoca melhoria dos parâmetros hemodinâmicos, efeito diurético e altera favoravelmente o perfil neurohumoral, tendo já sido aprovada a sua utilização na descompensação aguda da insuficiência cardíaca pela "Food & Drug Administration"[146-152]. O ANP (carperitide) endovenoso apresenta efeitos semelhantes ao do nesiritide e foi também aprovado no Japão para o tratamento da insuficiência car-

díaca aguda[153]. Encontra-se ainda em desenvolvimento a urodilatina (ulatiride), para aplicação nessa mesma situação. No entanto, o facto da utilização terapêutica dos PN implicar o uso da via endovenosa torna a sua aplicação em doentes com insuficiência cardíaca crónica não exequível. Um estudo em animais tratados com BNP por via subcutânea demonstrou a sua eficácia na melhoria dos parâmetros hemodinâmicos, sugerindo a possibilidade da utilização desta técnica em humanos[154].

A administração de inibidores da endopeptídase neutra (candoxatril) aumenta as concentrações dos PN cardíacos e, em doentes com insuficiência cardíaca, aumenta a excreção de sódio e diminui as pressões auriculares direitas e esquerdas[155-158]. No entanto, a NEP é responsável pela degradação de outras substâncias para além dos PN, como a angiotensina II e a endotelina 1, que actuam como vasoconstritores. Assim, o efeito dos inibidores da NEP depende largamente do seu efeito preferencial sobre os substratos vasodilatadores ou vasoconstritores dessa enzima. Este fenómeno foi ultrapassado pelo desenvolvimento dos inibidores da vasopeptídase, fármacos com capacidade de inibição da endopeptídase neutra e da enzima conversora da angiotensina. O omapatrilat foi o primeiro fármaco desta classe, activo por via oral, a ser introduzido em ensaios clínicos. Apesar dos estudos iniciais na insuficiência cardíaca mostrarem melhoria dos parâmetros hemodinâmicos e neurohumorais e sugerirem a superioridade do omapatrilat sobre o lisinopril[159-162], estes resultados não foram confirmados no ensaio clínico OVERTURE, no qual não se registaram diferenças no evento morte/hospitalização por insuficiência cardíaca entre o grupo medicado com omapatrilat e o grupo sob terapêutica com enalapril[163].

1.2. OS ESTADOS DE HIPERVOLÉMIA

Os estados de hipervolémia caracterizam-se por uma retenção inadequada de sal e água, conduzindo à expansão dos compartimentos hídricos do organismo, nomeadamente do volume plasmático e do volume extracelular.

Na insuficiência renal, a perda da capacidade funcional dos rins determina a diminuição da excreção de sódio e água e consequentemente a expansão de volume intravascular e extravascular, que geralmente só se manifesta nos estádios mais avançados dessa patologia.

Já nos restantes estados de hipervolémia como a insuficiência cardíaca, a cirrose e a gravidez, a capacidade funcional dos rins está geralmente preservada. No entanto, ocorre retenção de sódio e água pelo rim apesar de se verificar expansão da volémia, de forma aparentemente paradoxal.

Em 1988, Schrier propôs a explicação para este e outros achados aparentemente contraditórios nos estados de hipervolémia[164-167]. Segundo este investigador o marco comum a estas patologias será o subpreenchimento arterial ("arterial underfilling"), que despoletará uma série de eventos subsequentes destinados a restaurar a integridade circulatória arterial. Assim, a redução do débito cardíaco (no caso da insuficiência cardíaca de baixo débito) ou a vasodilatação arterial periférica (no caso da cirrose, insuficiência cardíaca de alto débito e gravidez) causarão um subpreenchimento arterial, que, por sua vez, induzirá a retenção de sódio e água e o aumento das resistências vasculares periféricas (no 1º caso) ou o aumento do débito cardíaco (no 2º caso), tendo como objectivo restaurar o preenchimento arterial. Isto acontecerá apesar do volume total de sangue se encontrar aumentado, porque cerca de 85% do sangue se encontra no lado venoso da circulação, enquanto no arterial se encontram apenas os 15% restantes. Deste modo, o subpreenchimento do compartimento arterial será o elo patofisiológico comum à retenção de sódio e água que ocorre paradoxalmente nos estados de hipervolémia e que resulta nas manifestações congestivas a eles associadas (edema periférico e pulmonar, ascite).

Na insuficiência cardíaca ocorre um diminuição progressiva do débito cardíaco por falência cardíaca primária, acompanhada de um aumento progressivo dos sistemas neurohumorais compensatórios (renina-angiotensina-aldosterona, adrenérgico e arginina vasopressina). Na cirrose hepática é actualmente aceite a "hipótese da vasodilatação periférica", como a explicação para a diminuição das resistências vasculares periféricas e da tensão arterial, o aumento do débito cardíaco e a estimulação neuro-humoral encontradas nessa patologia[168].

O subpreenchimento arterial é detectado por mecano-receptores ventriculares, no seio carotídeo, arco aórtico e arteríolas aferentes renais, resultando na estimulação do tónus simpático, do sistema renina-angiotensina-aldosterona e da sede e na libertação não-osmótica de vasopressina (um mecanismo menos sensível mas mais potente do que a regulação osmótica)[169,170]. A libertação não-osmótica de vasopressina explica a ausência de supressão desta hormona verificada em situações de hipervolémia e hipo-osmolalidade como as que ocorrem na insuficiência cardíaca e cirrose avançadas[171,172].

Em indivíduos normais, a administração de níveis elevados de aldosterona conduz apenas a uma retenção de sódio limitada e não condiciona a formação de edema, ilustrando o mecanismo denominado de "escape à aldosterona". Nos indivíduos com insuficiência cardíaca ou cirrose, a administração de aldosterona condiciona agravamento de edema pulmonar e ascite, ou seja, não existe "escape à aldosterona". O local de acção da aldosterona no rim é o tubo colector, na porção terminal do nefrónio. Quer uma diminuição na taxa de filtração glomerular, quer um aumento na reabsorção tubular proximal de sódio podem resultar na diminuição do aporte distal de sódio e água, condicionando a acção da aldosterona. Como o escape à acção retentora de sódio da aldosterona é dependente do aumento do aporte de sódio ao nefrónio distal, as respostas ao subpreenchimento arterial podem causar diminuição desse aporte, explicando a perda do escape à aldosterona.

Tal como a aldosterona, também os efeitos diurético e natriurético dos PN dependem do aporte de sódio ao tubo colector no nefrónio distal e, portanto, estarão condicionados pelo subpreenchimento arterial. Será este um dos mecanismos subjacentes à resistência aos PN encontrada nas situações de insuficiência cardíaca e cirrose hepática[170].

1.3. OS PEPTÍDEOS NATRIURÉTICOS NOS ESTADOS DE HIPERVOLÉMIA

O facto dos PN, nomeadamente do ANP e do BNP, serem hormonas responsáveis pela homeostasia hidro-electrolítica levou a que se colocasse a hipótese de existir deficiência desses peptídeos nos estados de hipervolémia. Os primeiros doseamentos de

ANP em doentes com insuficiência cardíaca e cirrose vieram contrariar essa hipótese, mostrando que os níveis de PN estão aumentados nessas circunstâncias[173]. De igual modo, foram documentadas concentrações elevadas de PN nos doentes com insuficiência renal[174-176].

Assim, nos estados de hipervolémia parece haver activação do sistema dos PN, embora a(s) causa(s) dessa activação estejam ainda por elucidar cabalmente. Para além da sobrecarga de pressão e volume resultante da expansão plasmática característica desses estados, também a activação neuro-humoral comum à insuficiência cardíaca, insuficiência renal e cirrose, a presença de alterações cardíacas intrínsecas ou uma deficiência nos mecanismos de eliminação/degração dos PN, poderiam explicar os níveis plasmáticos aumentados dos PN.

Na insuficiência cardíaca e na cirrose parece ainda existir um estado de resistência aos efeitos dos PN. Enquanto em indivíduos normais a administração de ANP exógeno condiciona aumento da diurese e da excreção de sódio, nos doentes com cirrose ou insuficiência cardíaca há resistência renal à hormona, com atenuação do seu efeito[177-180].

Foram aventadas várias explicações para a resistência/atenuação dos efeitos diuréticos e natriuréticos dos PN na insuficiência cardíaca[181], nomeadamente a redução dos receptores renais ou o aumento de actividade da NEP no túbulo proximal, impedindo a chegada do ANP/BNP ao seu local de acção no tubo colector. Estas hipóteses são afastadas por estudos que demonstram a presença aumentada de GMPc, o 2º mensageiro dos PN, na urina de doentes com insuficiência cardíaca e na cirrose[182,183]. O factor mais importante para a resistência aos PN nestas circunstâncias deverá ser a diminuição no aporte de sódio e água ao túbulo distal, de forma semelhante ao que ocorre com a aldosterona.

A resistência aos PN parece, no entanto, ser característica das fases avançadas da insuficiência cardíaca[184]. Nas fases mais iniciais da doença, o aumento dos PN condiciona uma resposta diurética e natriurética.

Tal como na insuficiência cardíaca, também na cirrose parece existir resistência aos PN, provavelmente também em consequência da diminuição do aporte distal de sódio e água[179,180,185].

Em suma, os estados edematosos, quer os consequentes à perda da função excretora de sódio e água, como a insuficiência renal, quer os resultantes da retenção inapropriada de sódio e água activada pelo subpreenchimento arterial, como a insuficiência cardíaca e a cirrose, parecem cursar com níveis aumentados de PN. As causas e as consequências dessa activação permanecem por elucidar. O esclarecimento da associação entre PN e estados de hipervolémia poderia ter implicações clínicas. Assim, uma putativa associação entre BNP ou NT-próBNP e a volémia ou alterações cardíacas intrínsecas nessas situações poderia ser útil na sua monitorização terapêutica e avaliação prognóstica.

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II. Objectivos

Com o conjunto de trabalhos que compõem esta dissertação pretendemos estudar a expressão do sistema dos PN, nomeadamente do BNP e do NT-próBNP, em várias entidades clínicas caracterizadas pela retenção inapropriada de sódio e água e estimar as implicações clínicas dela resultantes.

Constituíram objectivos específicos dos estudos:

- Avaliar a activação do sistema dos peptídeos natriuréticos, nomeadamente da síntese de BNP/NT-próBNP no estados de hipervolémia: insuficiência cardíaca, cirrose hepática e insuficiência renal
- Identificar preditores das concentrações plasmáticas de BNP/NT-próBNP na insuficiência cardíaca aguda, na insuficiência renal e na cirrose hepática, particularmente entre marcadores de função cardíaca e de volémia
- Determinar o valor do BNP/NT-próBNP como marcador prognóstico na estratificação de risco nos estados de hipervolémia: insuficiência cardíaca, cirrose hepática e insuficiência renal.

III. Métodos

ESTUDO 1

"Amino-terminal B-type natriuretic peptide, renal function, and prognosis in acute heart failure: a hospital cohort study". *J Card Fail* 2007;13:275-280.

Doentes: Neste trabalho foram avaliados 511 doentes internados por descompensação de insuficiência cardíaca entre Outubro de 2002 e Abril de 2004. Foram excluídos os doentes com síndromas coronárias agudas, os doentes em hemodiálise, os que faleceram no internamento e os que não tinham quantificação de NT-próBNP disponível à admissão e alta, ficando disponíveis para análise os dados de 283 doentes.

Métodos: As concentrações de NT-próBNP à admissão e alta foram determinadas por imunoensaio de quimioluminescência (Roche Diagnostics). Com base na variação do NT-próBNP durante a hospitalização, classificámos os doentes em 2 grupos: (1) com descida de NT-próBNP em resposta à terapêutica (descida de NT-próBNP≥30% do valor da admissão; (2) sem resposta dos níveis de NT-próBNP (descida de NT-próBNP<30% ou aumento do seu valor). As concentrações plasmáticas de creatinina, ureia e albumina foram quantificadas segundo os métodos habituais no laboratório hospitalar e estimámos a taxa de filtração glomerular (TFGe) utilizando a equação do estudo MDRD ("modification of diet in renal disease"). De acordo com a TFGe, dividimos os doentes em 4 grupos: (1) TFGe normal (TFGe ≥90 mL/min/1.73 m^2), (2) diminuição ligeira da TFGe (TFGe 60-89 mL/min/1.73 m^2), (3) diminuição moderada da TFGe (TFGe 30-59 mL/min/1.73 m^2), e (4) diminuição grave da TFGe (TFGe <30 mL/min/1.73 m^2).

Foi efectuado seguimento dos doentes nos 6 meses após a alta hospitalar, para determinar a utilidade do NT-próBNP como marcador de sobrevida livre de internamento de qualquer causa, quer nos doentes com função renal preservada, quer no grupo de doentes com diminuição da TFGe.

ESTUDO 2

"Aminoterminal B-type natriuretic peptide (NT-proBNP) in end-stage renal failure patients on regular hemodialysis: does it have diagnostic and prognostic implications?" *Nephron Clin Pract* 2009;111:182-188.

Doentes: Para este estudo recrutámos 44 doentes com insuficiência renal crónica terminal em programa regular de hemodiálise.

Métodos: Foram colhidas amostras de sangue imediatamente antes e no final de uma sessão de hemodiálise. Quantificámos os níveis de NT-próBNP utilizando um imunoensaio de quimioluminescência (Elecsys 2010, Roche Diagnostics). Calculámos a *ratio* de redução de NT-próBNP associada à sessão de hemodiálise pela fórmula: (NT-próBNP pré-hemodiálise – NT-próBNP pós-hemodiálise)/NT-próBNP pré-hemodiálise x 100. Os restantes parâmetros analíticos foram determinados segundo os métodos habituais do laboratório. Registámos o volume de ultrafiltração e a variação de peso intradialítica e interdialítica. Avaliámos os parâmetros cardíacos obtidos por ecocardiograma e calculámos a massa ventricular utilizando a fórmula de Devereux e Reichek: Massa ventricular (g) = {1.04 x [(diâmetro telediastólico do ventrículo esq + espessura do septo interventricular + espessura da parede posterior)^3 – (diâmetro telediastólico do ventrículo esquerdo)^3]} – 13.6, normalizada para a altura$^{2.7}$.

Os doentes foram seguidos prospectivamente durante 656,5±476,4 dias, avaliando-se a ocorrência do evento combinado morte/hospitalização de qualquer causa.

ESTUDO 3

"BNP at discharge in acute heart failure patients: Is it all about volemia? A study using impedance cardiography to assess fluid and hemodynamic status." *Int J Cardiol* 2009. doi:10.1016/j.ijcard.2009.08.001 [Epub ahead of print]

Doentes: Avaliámos uma amostra de conveniência de 163 doentes internados por insuficiência cardíaca aguda, nos quais foi excluída a presença de síndroma coronária aguda, doença valvular moderada a grave ou presença de próteses aórticas.

Métodos: No dia da alta hospitalar foi efectuado a todos os doentes um exame de cardiografia de impedância utilizando um Monitor Niccomo (Cardiodynamics, San Diego, Califórnia) para determinar o conteúdo de fluído torácico e parâmetros hemodinâmicos. Este dispositivo utiliza 2 pares de sensores (cada um com 2 eléctrodos) colocados a 180º na base do pescoço e na linha médio axilar ao nível da apófise xifóide. Os eléctrodos externos transmitem uma corrente de baixa amplitude e alta frequência (2.5 mA, 70 kHZ) e os eléctrodos internos detectam alterações na voltagem, que são usadas para calcular alterações na impedância. A impedância torácica basal é um indicativo do conteúdo de fluído no tórax e o recíproco desse parâmetro é denominado conteúdo de fluído torácico. A impedância dinâmica é influenciada pelo volume e velocidade na aorta e as alterações na impedância torácica são processadas, permitindo calcular o volume de ejecção e o débito cardíaco; os outros parâmetros hemodinâmicos são calculados utilizando equações-padrão. Foram obtidas amostras de sangue à data da alta e foi quantificada a concentração de BNP utilizando um imunoensaio de quimioluminescência (Abbot AxSYM). As outras análises laboratoriais foram efectuadas segundo os métodos habituais no laboratório do hospital.

Após a alta, seguimos prospectivamente os doentes durante 2 meses, avaliando a ocorrência de morte ou hospitalização de qualquer causa.

ESTUDO 4

"B-type natriuretic peptide is related to cardiac function and prognosis in hospitalized patients with decompensated cirrhosis." *Liver Int* 2010. doi:10.1111/j.1478-3231.2010.0226.x [Epub ahead of print]

Doentes: Estudámos 91 doentes com cirrose hepática descompensada, internados consecutivamente durante um ano. Oito doentes com história de insuficiência cardíaca foram excluídos.

Métodos: Procedemos à realização de entrevista clínica, exame físico, colheita de amostras de sangue para quantificação de BNP e outros parâmetros analíticos e estudo de cardiografia de bioimpedância na admissão. Avaliámos do mesmo modo um grupo de 13 controlos saudáveis com distribuição etária e por género semelhante à dos doentes. A cardiografia de bioimpedância e o doseamento de BNP foram efectuados como descrito previamente no estudo 3.

Os doentes que tiveram alta hospitalar foram seguidos prospectivamente durante 6 meses, para avaliar a ocorrência de morte de qualquer causa.

IV. Publicações

Journal of Cardiac Failure Vol. 13 No. 4 2007

Amino Terminal B-Type Natriuretic Peptide, Renal Function, and Prognosis in Acute Heart Failure: A Hospital Cohort Study

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ABSTRACT

Background: Amino-terminal pro-brain natriuretic peptide (NT-proBNP) is a valuable diagnostic and prognostic test in heart failure (HF). Limited information is available concerning its use in patients with renal failure, in whom dependence on renal clearance may negatively affect its performance.
Methods and Results: We evaluated influence of renal function on NT-proBNP levels and on its prognostic value after hospital discharge in 283 acute HF patients. Admission and discharge NT-proBNP levels were higher in patients with decreased estimated glomerular filtration rate (eGFR). In these patients discharge NT-proBNP above median was associated to occurrence of death or readmission at 6 months (hazard ratio [HR] 2.53, 95% confidence interval [CI] 1.27−5.03); in patients with normal eGFR, a trend to this association was found (HR 1.64, CI 0.98−2.76). Decrease in NT-proBNP less than 30% of baseline was associated to outcome in patients with normal eGFR (HR 2.68, CI 1.54−4.68) and decreased eGFR (HR 2.54, CI 1.49−4.33).
Conclusions: Acute HF patients with renal failure have higher NT-proBNP levels than those with normal renal function. Discharge NT-proBNP has long-term prognostic value in HF patients with renal dysfunction. NT-proBNP variations during hospitalization provide additional prognostic information either in patients with normal or reduced eGFR. (*J Cardiac Fail 2007;13:275−280*)
Key Words: NT-proBNP, heart decompensation, renal failure, event-free survival.

Impairment of renal function has been increasingly recognized in heart failure (HF) patients.[1−3] Renal involvement in HF is multifactorial and many risk factors are shared by HF and renal failure. On the other hand, patients with chronic kidney disease are at increased risk of cardiovascular disease, including its end-stage condition, HF.[4−6] The interaction between HF and renal failure can lead to deterioration of both conditions and is associated with progression of HF syndrome. This recognized ''cardio-renal'' association has diagnostic, pathophysiologic, therapeutic, and prognostic implications.[7−9]

Natriuretic peptides have become valuable instruments helping clinicians in HF diagnosis and risk stratification. Their application in chronic kidney disease patients might nevertheless have some constraints, as an elevation of natriuretic peptides has been consistently reported in that context, correlated with the reduction of glomerular filtration rate.[2,10−12] The performance of brain natriuretic peptide (BNP) and its amino-terminal pro-fragment (NT-proBNP) in HF diagnosis according to renal function has now been evaluated. Different studies showed that BNP and NT-proBNP concentrations are elevated in proportion to renal impairment in HF patients.[2,3,13] Nevertheless, BNP and NT-proBNP maintain their accuracy in the diagnosis of acute HF in patients with renal dysfunction although higher best decision values may have to be considered.[2,3,13] These data reinforce the postulate that BNP and NT-proBNP mirror the cardio-renal interaction more than exclusively cardiac derangement. We aimed to evaluate the influence of renal function on NT-proBNP levels and the prognostic value of NT-proBNP after hospital discharge for an episode of acute HF.

Patients and Methods

Patients

We included patients admitted between October 2002 and April 2004 to our department because of decompensated HF, defined as

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Manuscript received June 27, 2006; revised manuscript received December 28, 2006; revised manuscript accepted January 4, 2007.
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1071-9164/$ - see front matter
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doi:10.1016/j.cardfail.2007.01.001

exacerbation of symptoms in patients with at least 1 New York Heart Association class deterioration. The diagnosis of HF was based on the European Society of Cardiology criteria.[14] Patients with acute coronary syndromes and on hemodialysis were excluded.

Blood samples were collected in EDTA-containing tubes up to 24 hours after admission and before discharge. NT-proBNP was measured using a chemiluminescent immunoassay kit (Roche Diagnostics) on an Elecsys 2010 analyzer—measuring range 5 to 35000.0 pg/mL. The intra-assay coefficient of variation is 0.9% at mean values of 474 pg/mL, 1.1% at mean values of 8005 pg/mL, and 0.9% at mean values of 13682 pg/mL.

In analysis of the change in NT-proBNP during hospitalization, patients were classified into 2 groups: (1) those whose NT-proBNP decreased with therapy (NT-proBNP decreased by at least 30% of the baseline level) and (2) those with no response of NT-proBNP to therapy (NT-proBNP decreased less than 30% of baseline value or increased). This subdivision of patients according to NT-proBNP variations during hospitalization was based on previous observations indicating that patients not achieving a 30% decrease in NT-proBNP during hospital stay had a fourfold increase in the risk of adverse events.[15]

Serum creatinine, urea nitrogen and albumin were measured at hospital laboratory using standard methods. Estimated glomerular filtration rate (eGFR) was calculated using modification of diet in renal disease study equation:[16] $eGFR = 170 \times [\text{serum creatinine}]^{-0.999} \times [\text{age}]^{-0.176} \times [0.762 \text{ if patient is female}] \times [1.180 \text{ if patient is black}] \times [\text{serum urea nitrogen}]^{-0.170} \times [\text{serum albumin}]^{0.318}$.

Patients were classified according to eGFR into 4 groups: (1) normal eGFR (eGFR $\geq$90 mL/min/1.73 m^2), (2) mild decrease in eGFR (EGFR 60−89 mL/min/1.73 m^2), (3) moderate decrease in eGFR (eGFR 30−59 mL/min/1.73 m^2), and (4) severe decrease in eGFR (EGFR $<$30 mL/min/1.73 m^2).

Systolic dysfunction was defined as left ventricle ejection fraction $\leq$45% or by qualitative assessment of left ventricle function on echocardiography.

Patients received standard treatment with diuretics (furosemide with or without spironolactone), angiotensin-converting enzyme inhibitors and β-blockers according to attending physicians. For study analysis, we considered β-blockers and angiotensin-converting enzyme inhibitors dosage as follows: 10 mg bisoprolol = 50 mg carvedilol and 150 mg captopril = 30 mg lisinopril. Physicians were blinded to NT-proBNP levels.

Patients were followed for 6 months. The primary end point was all-cause death or readmission. Follow-up was made by telephone contact with patients or relatives, by an investigator blinded to NT-proBNP levels. The study was approved by the local ethics committee and patients gave informed consent.

We stored and analyzed data using SPSS (SPSS Inc, Chicago, Illinois). Changes in continuous variables were compared using the Wilcoxon test. The chi-square test was used to compare proportions among the 4 groups defined by eGFR. Differences in continuous variables between these classes of patients were tested using analysis of variance. Survival curves were estimated according to the Kaplan-Meier method, and compared by the log-rank test. The association of independent variables with time to outcome was assessed by Cox regression and is expressed as hazard ratio and 95% confidence interval. Unless otherwise noted, results are presented as median (interquartile range) for numeric variables and as n (%) for categorical variables. A significance level of 5% was used.

Results

During the study period, 511 patients were admitted because of decompensated HF, 52 (10.2%) of whom died in the hospital. Among the 419 patients discharged alive, NT-proBNP levels at admission and discharge were available for 287 patients. Of these, 4 were previously or became on dialysis during hospitalization and were excluded. The subsequent analysis refers to the other 283 patients [mean age = 72.8 ± 11.7 years; 147 (51.9%) females]. Table 1 shows demographic, clinical, functional, and laboratory characteristics of patients, according to eGFR class. The proportion of patients with renal failure, as defined by eGFR $<$90 mL/min/1.73 m^2, was 42.0%.

NT-proBNP levels decreased significantly during hospitalization both in patients with normal eGFR (change in NT-proBNP = 2574.1 ± 6452.7 pg/mL, $P < .001$) and in patients with renal failure (change in NT-proBNP = 7288.8 ± 27304.9 pg/mL, $P = .004$).

Table 2 presents treatment during hospitalization and at discharge in patients grouped by eGFR classes.

The median follow-up was 182 days (range 181−182 days). The combined end point of death or readmission occurred in 125 patients (43.5%) (61 [37.7%] patients with normal eGFR, 33 [44%] patients with mild decrease in eGFR, 22 [66.7%] patients with moderate decrease in eGFR, and 5 [50%] patients with severe decrease in eGFR). Time to the first event was used as dependent variable in survival analysis. There was a positive association between eGFR $<$90 mL/min/1.73 m^2 and time to readmission or death (HR 1.54; 95% CI, 1.08−2.20).

Admission NT-proBNP levels were not associated with outcome, as evaluated by Kaplan-Meier method, neither in the overall group (log-rank test, $P = .90$) nor in patients with and without renal failure, analyzed separately (log-rank test, $P = .60$ and $P = .68$, respectively).

Figure 1 represents the cumulative incidence of death or readmission according to discharge NT-proBNP levels (dichotomized according to the median), estimated by the Kaplan-Meier method, in patients with and without renal failure. Figure 2 shows Kaplan-Meier cumulative hospitalization-free survival curves according to the change in NT-proBNP levels during hospitalization for the four eGFR groups.

The association between NT-proBNP variation and NT-proBNP levels at discharge and survival was independent of age, sex, hemoglobin, serum sodium, left ventricle systolic function, systolic blood pressure, heart rate, and New York Heart Association class at discharge both in patients with and without renal failure as evaluated by multivariate Cox regression analysis (Table 3).

Discussion

We found in a population of acute HF patients that NT-proBNP levels were proportional to renal function

Table 1. Characteristics of Patients According to Estimated Glomerular Filtration Rate

	>90 mL/min/ 1.73 m^2 (n = 164)	60−89 mL/min/ 1.73 m^2 (n = 75)	30−59 mL/min/ 1.73 m^2 (n = 34)	<30 mL/min/ 1.73 m^2 (n = 10)	*P*
Age (y)	70.4 ± 12.4	75.6 ± 9.9	77.9 ± 8.6	72.5 ± 11.9	<.001
Female, n (%)	63 (38.4)	52 (69.3)	24 (70.6)	8 (80.0)	<.001
NYHA Class III/IV at discharge, n (%)	15 (12.4)	9 (15.8)	6 (24)	1 (10.0)	.50
Ischemic etiology, n (%)*	80 (67.8)	34 (56.7)	20 (66.7)	6 (60.0)	.19
Diabetes mellitus, n (%)	61 (38.1)	36 (48.0)	15 (46.9)	7 (70.0)	.14
Hypertension, n (%)	71 (44.4)	43 (57.3)	25 (75.8)	9 (90.0)	.001
Atrial fibrillation, n (%)	68 (42)	34 (47.9)	14 (43.8)	3 (30.0)	.60
Systolic dysfunction, n (%)*	94 (73.4)	45 (80.4)	16 (84.2)	2 (33.3)	.03‡
Ventricular function not assessed	36 (22.0)	19 (25.3)	15 (44.1)	4 (40.0)	
Systolic blood pressure at admission, mm Hg	122 (110−143)	120 (106−150)	130 (118−151)	155 (138−171)	.02
Heart rate at admission, bpm	90 (81−104)	90 (74−100)	89 (80−97)	100 (87−111)	.08
Hemoglobin at admission (g/dL)	12.7 (11.2−14.4)	11.8 (10.0−13.5)	11.5 (9.8−12.4)	11.5 (8.6−12.0)	.002
Sodium at discharge (mEq/L)	140 (137−142)	140 (137−141)	140 (138−142)	138 (134−144)	.44
Sodium ≤135 mEq/L at discharge, n (%)	20 (12.2)	13 (17.3)	6 (17.6)	6 (42.9)	.05
NT-proBNP at admission (pg/mL)	4807 (2089−9847)	10578 (4538−20416)	10776 (5342−31264)	17789 (10639−43691)	<.001
NT-proBNP at discharge (pg/mL)	2575 (1232−6454)	5512 (2223−11002)	7504 (4120−17592)	25010 (2785−37747)	<.001
NT-proBNP decrease ≥30%, n (%)	88 (53.7)	43 (57.3)	17 (50.0)	4 (40.0)	.72

All continuous variables are given as median (interquartile range) except for age, expressed as mean (SD).
NYHA, New York Heart Association; NT-proBNP, amino-terminal pro-brain natriuretic peptide.
*Data are proportions of patients with a measurement of the relevant variable.
‡Versus preserved systolic function.

impairment. In addition, we observed that discharge NT-proBNP was a useful marker of long-term prognosis in patients with renal dysfunction and also that NT-proBNP variations during hospitalization predicted readmission and death even in patients with mild/moderate renal dysfunction.

The prognostic value of NT-proBNP has been extensively reported. It has been observed in all spectrum of HF severity and in ambulatory and acute setting.[17−20] Despite these extensive data, limited information is available in HF patients with renal impairment. Moreover, it has been suggested that NT-proBNP diagnostic and prognostic performance might be negatively affected by decreased renal function.[13,21] Data from the ProBNP Investigation of Dyspnea in the Emergency Department (ie, PRIDE) study demonstrate that NT-proBNP measured in dyspneic patients in emergency department has short-term prognostic value even in patients with mild to moderate renal failure.[3] Recently, the more comprehensive International Collaborative on NT-proBNP (ICON) study, including patients from PRIDE and other centers, with a significant prevalence of renal failure, confirmed NT-proBNP as a strong predictor of 60-day mortality in acute HF patients in the presence of normal or impaired renal function.[22] But most studies on natriuretic peptides have excluded patients with moderate to severe renal failure. In our study, renal dysfunction was quite common, with a prevalence of eGFR <90 mL/min/1.73 m^2 of 42% (of these 37% moderate to severe renal failure), confirming that cardiac and renal insufficiencies are often coincidental and that ''cardio-renal interaction'' is a clinically relevant concept. Those patients had a more adverse prognosis, as shown by a significant positive association between eGFR <90 mL/min/1.73 m^2 and time to readmission or death. This finding stresses the importance of risk stratification in this subgroup of patients. Our study provides evidence that value of NT-proBNP as a prognostic marker is also observed in acute HF patients with renal failure.

Whether admission or discharge NT-proBNP concentrations are more accurate as a prognostic tool in acute HF

Table 2. Pharmacologic In-Hospital and Discharge Treatment of Patients According to Estimated Glomerular Filtration Rate

	≥90 mL/min/1.7 3 m^2 (n = 164)	60−89 mL/min/1.73 m^2 (n = 75)	30−59 mL/min/1.73 m^2 (n = 34)	<30 mL/min/1.73 m^2 (n = 10)	*P*
In-hospital treatment					
Furosemide, mg/d	80 (60−80)	80 (60−115)	80 (80−120)	120 (80−120)	.12
Lisinopril-equivalent, mg/d	10 (5−15)	10 (5−10)	10 (5−20)	5 (2.5−12.5)	.69
Carvedilol-equivalent, mg/d	12.5 (6.25−12.5)	12.5 (6.3−12.5)	6.25 (6.3−12.5)	12.5 (6.3−50)	.13
Spironolactone, mg/d	25 (12.5−25)	16.5 (12.5−25)	12.5 (12.5−18.8)	12.5 (12.5−12.5)	.32
Discharge treatment					
Furosemide, mg/d	60 (40−80)	60 (40−80)	80 (40−110)	80 (45−120)	.20
Lisinopril-equivalent, mg/d	10 (5−20)	10 (5−20)	10 (5−20)	5 (5−10)	.75
Carvedilol-equivalent, mg/d	12.5 (12.5−15)	12.5 (10.2−21.9)	6.25 (6.3−15.6)	—	.16
Spironolactone, mg/d	25 (12.5−25)	25 (12.5−25)	18.75 (12.5−25)	—	.23

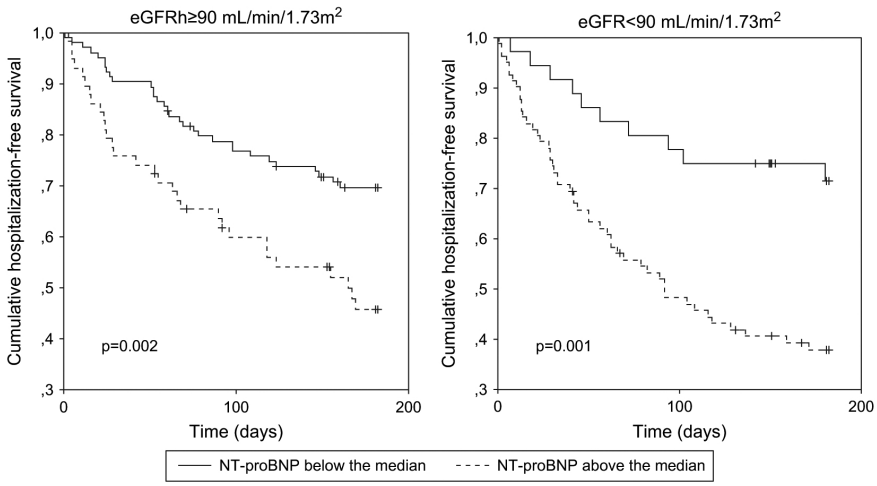


Fig. 1. Cumulative hospitalization-free survival according to NT-proBNP plasma level at discharge in HF patients with normal eGFR and decreased eGFR.

patients has been subject to same debate. We, and others, have previously reported that discharge NT-proBNP levels, but not admission, had strong prognostic value on that context.[23,24] Nevertheless, Januzzi et al reported that NT-proBNP measured at presentation in emergency department in acute HF patients was predictive of short-term mortality.[3] In our population, we did not find an association between admission NT-proBNP and outcome in either the overall group or in patients with and without chronic kidney disease. The issue remaining behind these apparent conflicting findings might be the different populations evaluated. In fact, the ICON population included acute HF patients that presented to the emergency department, an expectable proportion of them getting discharged home. On the other hand, other studies focused on hospitalized HF patients. This population is probably more volume-overloaded and that fact may account for a significant proportion of admission NT-proBNP concentration, the so called "wet NT-proBNP," that can be amenable by therapeutic intervention. Another possible factor linked to that observation is the noninclusion of patients that died during hospitalization.

In risk stratification and monitoring of HF patients, as suggested by us and others, probably serial measurements more than static values are meaningful.[23,25] The plasticity of the system and the possibility that therapeutic interventions lead to attenuation of natriuretic system activation evaluated by repeated determination of natriuretic peptide levels are probably the fulcrum of successful strategies. The present data on patients with renal dysfunction clearly reinforce this hypothesis. We observed that the deactivation of BNP, measured by a reduction on NT-proBNP levels for more than 30% between hospital admission and discharge is associated with better outcome in patients with and without renal dysfunction. Putting the focus on the group of patients with renal dysfunction, the relation between NT-proBNP levels' variation and prognosis was observed in all classes of renal failure except in those with the most severe impairment (eGRF <30 mL/min/1.73 m^2). Several factors may be related to this observation: (1) limited number of patients with severe renal failure; (2) the significantly higher admission NT-proBNP values of these patients, and (3) that for purposes of the analysis renal function was assessed at hospital discharge and these patients could have had significant deterioration of renal function during hospitalization that is associated with increasing natriuretic peptide levels.

Interestingly, the proportion of patients that exhibited a deactivation of natriuretic peptides system, reflected by a decrease in NT-proBNP $\geq$30%, was similar in all classes of eGFR and around 50%. This deactivation of natriuretic peptide system irrespective of renal function underlines the importance of serial measurement of NT-proBNP in assisting programming of hospital discharge, even in patients with diminished renal clearance, in whom absolute NT-proBNP levels are significantly higher. In this subset of patients, variation of NT-proBNP more than its absolute serum levels might have to be considered in risk stratification, especially if a single determination is made at admission. Moreover, the significant decrease of NT-proBNP concentrations during hospitalization even in patients with mild to moderate renal failure indicates that a considerable proportion of NT-proBNP levels in chronic kidney disease patients with HF is related to treatable volume expansion or neurohumoral manipulation, as the other proposed factors leading to increased NT-proBNP in renal failure, namely cardiac structural abnormalities and decreased renal clearance, supposedly remained unchanged during that period.

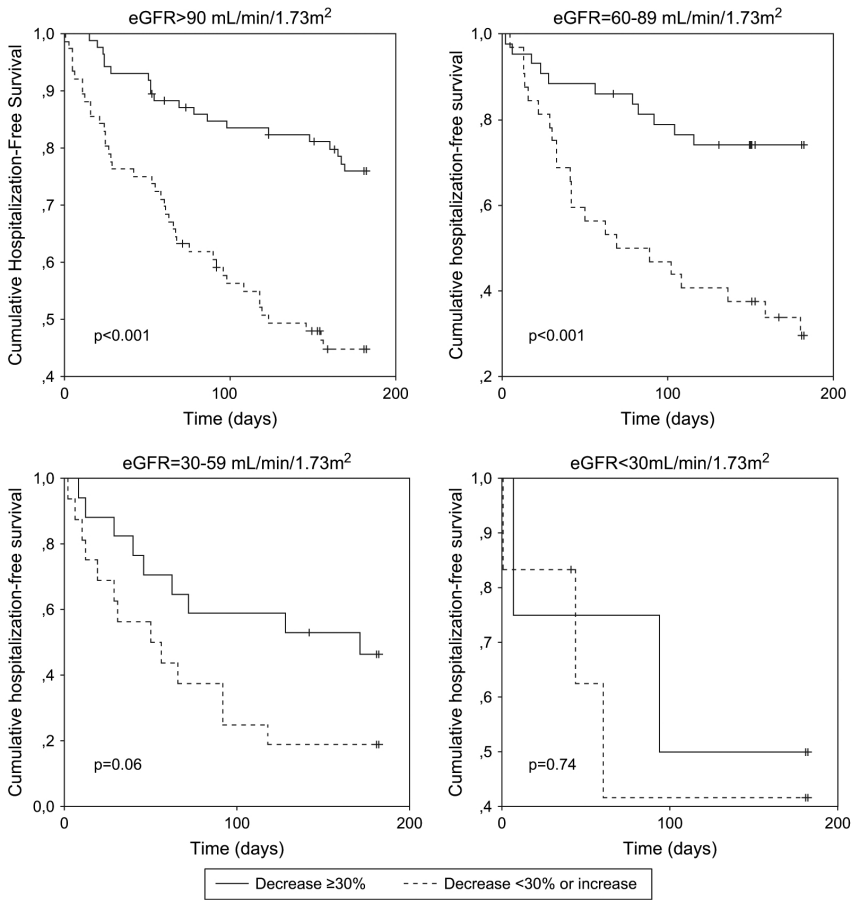


Fig. 2. Cumulative hospitalization-free survival according to patterns of variation of NT-proBNP (decrease ≥30% and decrease <30% or increase) in groups of HF patients with normally mild, moderate, and severe decrease in eGFR.

In conclusion, decompensated HF patients with renal dysfunction have higher levels of NT-proBNP at admission and discharge than those without renal dysfunction. Discharge NT-proBNP has prognostic value in patients with renal failure. The NT-proBNP variations during hospitalization provide additional prognostic information in patients with normal renal function as well as in patients with mild to moderate renal failure. Further studies are needed in patients with severe renal failure to explore the prognostic value of NT-proBNP in this special group of patients.

Limitations

Our study is a single-center study, requiring validation from other centers. Another limitation is that almost 50% of patients admitted because of acute HF during the study period did not have the information needed for analysis (and therefore were not included in the analysis), reinforcing the need for replication of these results. We excluded only renal failure patients on hemodialysis (the relation of NT-proBNP levels at admission and discharge with the technique could not be controlled and the effect of

Table 3. Multivariate Cox Regression Analysis for the Identification of Predictors of Death or Hospital Readmission After Discharge From Index Hospitalization Resulting From HF

	Death or Readmission HR (95% CI)
With eGFR ≥90 mL/min/1.73 m^2	
NT-proBNP at discharge above median	1.64 (0.98−2.76)
NT-proBNP decrease <30%	2.68 (1.54−4.68)
With eGFR <90 mL/min/1.73 m^2	
NT-proBNP at discharge above median	2.53 (1.27−5.03)
NT-proBNP decrease <30%	2.54 (1.49−4.33)

HF, heart failure; HR, heart rate; eGFR, Estimated glomerular filtration rate; NT-proBNP, amino-terminal pro-brain natriuretic peptide.

dialysis on NT-proBNP is not well known). The proportion of patients with moderate to severe renal dysfunction was smaller than previously reported in other studies of acute HF patients.[1−3] Possibly, this finding can be explained by the fact that we, unlike other authors, used discharge and not admission creatinine levels to estimate GFR, to minimize the transient deleterious effect on renal function of HF decompensation.

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Original Paper

nephron
Clinical Practice

Nephron Clin Pract 2009;111:c182–c188
DOI: 10.1159/000199458

Received: July 26, 2007
Accepted: September 25, 2008
Published online: February 5, 2009

Aminoterminal B-Type Natriuretic Peptide (NT-proBNP) in End-Stage Renal Failure Patients on Regular Hemodialysis: Does It Have Diagnostic and Prognostic Implications?

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Key Words
End-stage renal disease · Hemodialysis · Left ventricular hypertrophy · NT-proBNP · Prognosis

Abstract
Background: Amino-terminal B-type natriuretic peptide (NT-proBNP) quantification is a useful cardiovascular diagnostic and prognostic test. In renal failure its use may have constraints, as it depends on renal clearance. We aimed to evaluate the clinical usefulness of NT-proBNP in hemodialysis (HD) patients. ***Methods:*** We measured NT-proBNP levels in 44 patients before and after a regular high-flux HD. Cardiac parameters were evaluated by echocardiography. Patients were followed up for 656.5 ± 476.4 days for the occurrence of hospital admission or death. ***Results:*** NT-proBNP levels decreased after HD ($p < 0.0001$) without correlation with ultrafiltration volume, Kt/V index, weight decrease or pulse pressure decrease. Pre- and post-HD NT-proBNP were similar in patients with and without left ventricular hypertrophy. Pre-HD levels were not significantly associated with prognosis, probably due to a type 2 error; post-HD NT-proBNP and NT-proBNP reduction ratio (RR) were predictive of the outcome by Kaplan-Meier analysis, but only RR remained associated with prognosis on multivariate regression analysis (hazard ratio 0.96; 95% confidence interval 0.93–0.99). ***Conclusion:*** Our results do not support the use of NT-proBNP in the diagnosis of left ventricular hypertrophy or volume status in HD patients, but suggest its utility in prognostic stratification. HD-related variation in NT-proBNP was associated with prognosis.

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Introduction

Cardiac natriuretic peptides (NP), in particular B-type natriuretic peptide and its aminoterminal portion (NT-proBNP), have emerged as important diagnostic and prognostic markers in cardiovascular disorders. More recently, the relationship between NP and renal failure has been addressed. Conceptually, this association seems attractive, as renal insufficiency is related to circulatory volume expansion (known stimulator of NP release) and its therapy relies on promoting fluid removal (using diuretics or dialysis). On the other hand, renal failure patients have a high incidence of cardiovascular disease.

High levels of NP have consistently been found in patients with renal failure, correlated with glomerular filtration rate and more pronounced in end-stage renal disease (ESRD) [1–5]. This observation probably explains the worse performance of NP in heart failure diagnosis observed in

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1660–2110/09/1113–0182$26.00/0

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several clinical studies in patients with mild to moderate renal failure, implicating use of higher cut-off levels [1, 5, 6]. The role of NT-proBNP as prognostic marker in ESRD patients is still largely unexplored. Few previous studies demonstrated that NT-proBNP was predictive of all-cause death in renal failure patients on hemodialysis (HD) [7–9].

A reduction in ANP and BNP levels following HD has generally been reported, but there is some uncertainty regarding the effect of this technique on NT-proBNP plasmatic levels, with the studies available reaching conflicting results [8, 10–13].

We conducted a prospective study to evaluate the performance of NT-proBNP in identifying structural and/or functional cardiac impairment in ESRD patients, to clarify the influence of HD on NT-proBNP levels and explore the prognostic value of NT-proBNP in this population.

Patients and Methods

Forty-four patients with ESRD on regular HD (4 hours' sessions, 3 times weekly) were included. Patients were treated with high-flux helixone dializers – Fresenius Medical Care Fx60 (ultrafiltration coefficient = 46 ml/h·mm Hg) in 32 (72.7%) patients, Fx80 (ultrafiltration coefficient = 59 ml/h·mm Hg) in 10 (22.7%) and Fx100 (ultrafiltration coefficient = 73 ml/h·mm Hg) in 2 (4.6%); blood flow was usually 300 ml/min and the dialysate was bicarbonate buffered and contained 140 mEq/l sodium, 2 mEq/l potassium, 100 mg/dl glucose and 2.5, 3 or 3.5 mEq/l calcium. Body fluid was withdrawn and the ultimate goal was to reach dry weight, as clinically determined by an experienced nephrologist. Blood pressure, heart rate and body weight were recorded before and after the HD session. Proportional pulse pressure was calculated using the formula: (systolic blood pressure – diastolic blood pressure)/systolic blood pressure × 100.

Blood samples were obtained from the arteriovenous fistula or central venous catheter immediately before and at the end of a mid-week HD session. For NT-proBNP measurement, blood was collected into plastic tubes with K_3EDTA, centrifuged at 4,500 rpm during 15 min and plasma stored at –80°C until it was assayed using a chemiluminescent immunoassay kit (Elecsys 2010, Roche Diagnostics, Basel, Switzerland). We calculated the percent variation in NT-proBNP following HD–NT-proBNP reduction ratio (RR) using the formula: (pre-HD NT-proBNP – post-HD NT-proBNP)/pre-HD NT-proBNP × 100. Hemoglobin, albumin, urea, sodium, calcium and phosphorus analysis were made with standard methods. Calcium levels were corrected to albumin according to the formula: corrected calcium (mg/dl) = [0.8 × (4.0 – albumin)] + serum calcium.

All patients were submitted to a colour-Doppler, M-mode and 2D echocardiography study in a non-HD day. Left ventricular mass was calculated according to Devereux and Reichek [14] using the formula: LVM (g) = {1.04 × [(left ventricular end-diastolic diameter + interventricular septum thickness + posterior wall thickness)^3 – (left ventricular end-diastolic diameter)^3]} – 13.6. Left ventricular mass was normalized to height$^{2.7}$ [15], to eliminate the effect of any change in body water reflected in weight and body surface area. Left ventricular hypertrophy was defined as greater than 49 g/m$^{2.7}$ for men and 47 g/m$^{2.7}$ for women.

Patients were followed up from index HD day (04/06/2003) until death, renal transplant or 31/12/2006. The primary endpoint was all-cause death or hospitalization. Surveillance was made by direct contact with patients or relatives and continuous observation of medical records. The occurrence and cause of hospital admissions or death was confirmed by consulting clinical records, by an investigator blinded to NT-proBNP levels.

Statistical Analysis

Data was stored and analyzed using the Statistical Package for Social Sciences software (SPSS Inc., Chicago, Ill., USA). Differences in continuous variables between groups were compared by use of Wilcoxon test. The χ^2 test was used to compare proportions. The paired-samples t test was used to test for differences in continuous variables before and after HD. The Spearman's correlation was used to evaluate the association between continuous variables. Survival curves were estimated according to the Kaplan-Meier method and compared by the log-rank test. The association of independent variables with time to outcome was assessed by Cox regression and is expressed as hazard ratio (HR) and 95% CI. A significance level of 5% was used. Otherwise noted, results are presented as mean ± SD for numeric variables and as n (%) for categorical variables.

The study was approved by the local Ethics Committee, and all patients gave informed consent for participation.

Results

The study included 44 patients (55.86 ± 15.74 years, 23 females (52.3%)). Time on HD was 2.60 ± 4.34 years, median = 1.04 years). Fourteen patients had diabetes, 6 had ischemic heart disease and 42 had history of hypertension. Ten patients were on angiotensin-converting enzyme inhibitors, 4 on beta-blockers and 8 on calcium antagonists. Thirty patients had left ventricular hypertrophy. None of the patients had left ventricular systolic dysfunction.

Effect of Hemodialysis on NT-proBNP Levels

NT-proBNP levels decreased significantly after HD (from 2,706.1 ± 2,683.3 to 2,195.5 ± 2,253.4 pg/ml, $p < 0.0001$). The mean decrease in NT-proBNP was 21.4 ± 12.8%. No significant correlation between the NT-proBNP RR and the urea reduction ratio, Kt/v index, ultrafiltration volume, decrease in body weight or decrease in proportional pulse pressure was found. Table 1 displays the characteristics of patients grouped according to NT-proBNP RR above or below median (21.0%).

Effect of Left Ventricular Mass on NT-proBNP Levels

Patients with and without left ventricular hypertrophy had similar pre-HD NT-proBNP levels (2,624.0 ±

Table 1. Characteristics of patients with NT-proBNP RR above and below the median (21%)

	NT-proBNP RR >median	NT-proBNP RR <median	p
Pre-HD NT-proBNP, pg/ml	1,882.5 ± 1,927.4	3,529.7 ± 3,099.3	0.009
Post-HD NT-proBNP, pg/ml	1,285.0 ± 1,293.0	3,106.0 ± 2,643.6	0.001
Age, years	53.1 ± 14.8	58.6 ± 16.5	0.255
Male sex, n (%)	10 (45.5)	11 (50.0)	0.763
Pre-HD SBP, mm Hg	131.8 ± 23.4	138.7 ± 26.8	0.411
Pre-HD DBP, mm Hg	69.3 ± 12.3	72.9 ± 19.3	0.707
Post-HD SBP, mm Hg	120.1 ± 24.6	130.5 ± 24.5	0.127
Post-HD DBP, mm Hg	68.6 ± 11.4	67.6 ± 18.6	0.353
Pre-HD heart rate, bpm	73.2 ± 10.7	70.9 ± 9.4	0.481
Post-HD heart rate, bpm	74.4 ± 14.8	73.6 ± 13.5	0.906
Pre-HD body weight, kg	63.6 ± 12.8	66.3 ± 13.2	0.503
Post-HD body weight, kg	61.1 ± 12.3	63.6 ± 12.7	0.489
Increase in body weight, %	3.9 ± 1.4	4.0 ± 1.6	0.830
Ultrafiltration volume, ml	2,781.0 ± 1,042.1	3,040.5 ± 1,264.2	0.520
Kt/V	1.4 ± 0.2	1.3 ± 0.3	0.115
Urea reduction ratio, %	75.0 ± 6.0	71.0 ± 10.0	0.097
HD time, h	3.9 ± 0.3	3.5 ± 0.7	0.031
Hemoglobin, g/dl	12.3 ± 1.1	11.2 ± 1.5	0.005
Serum sodium, mEq/l	136.6 ± 3.6	138.1 ± 2.6	0.262
Serum albumin, g/l	3.9 ± 0.2	3.7 ± 0.3	0.506
Serum calcium, mg/dl	9.2 ± 0.7	8.9 ± 0.5	0.111
Serum phosphate, mg/dl	3.9 ± 1.2	3.9 ± 1.5	0.814
Calcium × phosphate	36.6 ± 12.5	35.3 ± 12.5	0.780
LVMI, g/m$^{2.7}$	61.0 ± 18.0	65.2 ± 12.8	0.681

SBP = Systolic blood pressure; DBP = diastolic blood pressure; LVMI = left ventricular mass index.

2,357.8 vs. 2,882.1 ± 3,371.3 pg/ml, respectively, p = 0.51), post-HD NT-proBNP levels (2,100.1 ± 1,932.2 vs. 2,399.8 ± 2,898.5 pg/ml, respectively, p = 0.55) and NT-proBNP RR (21.4 ± 11.4 vs. 21.3 ± 15.3%, respectively, p = 0.72). No correlations were found between pre-HD, post-HD or NT-proBNP RR levels and left ventricular mass index.

Effect of Volume Status in NT-proBNP Levels

Ultrafiltration volume, weight decrease following HD and interdialytic weight gain did not differ in patients with pre-HD NT-proBNP levels above versus below the median (2,882.4 ± 1,137.1 vs. 2,952.1 ± 1,204.6 ml, p = 0.85; 2.6 ± 1.0 vs. 2.6 ± 1.2 kg, p = 0.98, and 2.8 ± 1.3 vs. 2.6 ± 1.4 kg, p = 0.80, respectively) or in patients with post-HD levels above or below median (2,980.2 ± 1,140.8 vs. 2847.6 ± 1,195.6 ml, p = 0.76; 2.7 ± 1.1 vs. 2.5 ± 1.1 kg, p = 0.61, and 3.0 ± 1.4 vs. 2.5 ± 1.2 kg, p = 0.39, respectively). Correlations of NT-proBNP RR with markers of volume variations were not significant: rho = −0.21, p = 0.19 for ultrafiltration volume; rho = −0.16, p = 0.31 for decrease in body weight following HD, and rho = −0.16, p = 0.29 for interdialytic weight gain.

Prognosis

Patients were followed up for 656.5 ± 476.4 days (median = 596.5, 194.3–1,195.5). Twelve patients received a kidney transplant during the follow-up period and were censored at that time. The combined end-point of death or hospitalization occurred in 27 patients (61.4%). Time to first event was used as dependent variable in survival analysis.

Patients who suffered death or hospitalization had significantly higher post-HD NT-proBNP levels and lower NT-proBNP RR than the other patients (2,808.1 ± 2,591.8 vs. 1,222.5 ± 1,044.9 pg/ml, p = 0.02, and 17.9 ± 10.4 vs. 26.9 ± 14.5%, p = 0.01, respectively). Pre-HD NT-proBNP levels did not differ significantly between the two groups (3,370.4 ± 3,044.1 vs. 1,651.0 ± 1,537.9 pg/ml, p = 0.06).

Figure 1 displays the cumulative incidence of death or hospital admission according to pre- and post-HD NT-

Table 2. Univariate Cox regression analysis for the identification of predictors of death and/or hospital admission

	HR	95% CI
Male gender (vs. female)	0.65	0.30–1.40
Age	1.02	0.99–1.04
Pre-HD heart rate	0.99	0.95–1.03
Post-HD heart rate	1.02	0.99–1.05
Pre-HD systolic BP	1.01	0.99–1.03
Pre-HD diastolic BP	1.01	0.98–1.03
Post-HD systolic BP	1.00	0.99–1.02
Post-HD diastolic BP	0.97	0.93–1.01
Post-HD NT-proBNP	2.53	1.15–5.56
NT-proBNP RR	0.96	0.93–0.99
Haemoglobin	0.68	0.50–0.92
Serum albumin	0.19	0.36–1.01
Serum sodium	0.92	0.82–1.04
Serum calcium	0.37	0.16–0.88
Serum phosphate	0.54	0.79–1.57
Calcium × phosphate	1.0	0.97–1.04
Kt/V	0.41	0.12–1.46
LVMI	1.02	1.00–1.04
Diabetes mellitus	1.79	0.83–3.88
Ischemic heart disease	1.11	0.38–3.21
Hypertension	1.26	0.17–9.38
Time on HD, years	0.94	0.85–1.05
ACE inhibitors	1.13	0.48–2.67
Beta-blockers	2.01	0.68–5.92
Calcium antagonists	1.80	0.76–4.29

BP = Blood pressure; LVMI = left ventricular mass index; ACE = angiotensin-converting enzyme.

proBNP levels and NT-proBNP RR (dichotomized according to the median), estimated by the Kaplan-Meier method. Only the post-HD NT-proBNP and the percent decrease in NT-proBNP were significantly associated with time to an adverse event.

All variables that were significantly associated with a higher hazard of dying or being admitted to hospital (table 2) in univariate analysis were included in the final multivariate Cox regression model performed by a stepwise method, depicted in table 3. Only NT-proBNP RR and calcium remained as significant predictors of the occurrence of the primary end-point.

Twenty-two patients were alive and not submitted to kidney transplant by the end of follow-up (December 31, 2006). In these patients, the number of hospital admissions correlated positively with pre-HD NT-proBNP levels (rho = 0.45, p = 0.036) and post-HD NT-proBNP levels (rho = 0.50, p = 0.019) and negatively with NT-proBNP RR (rho = −0.645, p = 0.001).

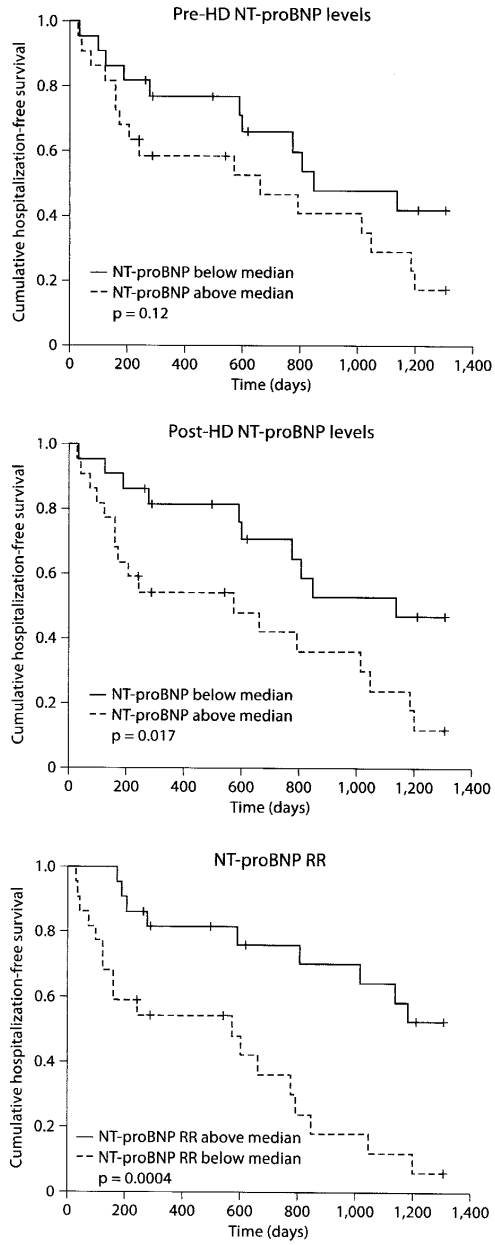


Fig. 1. Cumulative hospitalization-free survival according to pre-HD NT-proBNP levels (median, 1,602.5 pg/ml), post-HD NT-proBNP levels (median, 1,174.0 pg/ml) and NT-proBNP RR (median, 21%).

Table 3. Multivariate Cox regression analysis for the identification of predictors of death and/or hospital admission

	HR	95% CI
NT-proBNP RR	0.96	0.93–0.99
Serum calcium	0.37	0.15–0.91

Discussion

In this group of ESRD patients on regular HD without cardiac systolic dysfunction, we observed elevated NT-proBNP levels, exceedingly higher than the cut-off for heart failure diagnosis and not related to left ventricular mass. Hemodialysis induced a significant decrease in NT-proBNP levels, and the extent of this decrease was a better predictor of long-term event-free survival than absolute pre- or post-HD levels in this high-risk population.

Different studies showed that BNP and NT-proBNP are elevated in proportion to renal impairment in heart failure as well as in renal failure patients. Nevertheless, their utility in acute heart failure diagnosis is maintained in patients with mild-to-moderate renal dysfunction, although with higher best decision values of NP concentrations [1, 5, 6]. Our results do not expand these observations to ESRD patients but, on the contrary, suggest that NT-proBNP is not applicable to heart failure diagnosis in that population, due to their very high NT-proBNP levels. Furthermore, in this population with a high incidence of cardiovascular disorders, our findings contradict a potential value of NT-proBNP in identification of patients with left ventricular hypertrophy, although diagnostic performance could have been influenced by sample size. Recent studies have reported good NT-proBNP diagnostic performance for detecting left ventricle systolic dysfunction and increased LVMI in HD patients, but using considerably high cut-offs [16–18].

We observed a significant 21% decrease of NT-proBNP after HD, in consonance with similar studies measuring ANP, BNP or NT-proANP. The studies on NT-proBNP are conflicting. Clerico et al. [10] found a 9.5% rise of NT-proBNP following HD. Other authors reported a decrease in NT-proBNP of 18.4% using dialysis with high-flux membranes, and an increase in NT-proBNP in low-flux HD [11]. More recently, other study documented a 39% decrease in NT-proBNP using high-flux HD [8]. Our results extend these observations and suggest that NT-proBNP variations during HD must be evaluated according to dialysis membranes.

Several studies addressing the NP variation in response to volume removal through HD failed to establish a relationship between these two factors. We also could not find a correlation between ultrafiltration volume, decrease in body weight following HD or interdialytic weight gain and NT-proBNP RR. It thus seems that fluid removal and retention cannot account for the majority of NT-proBNP variation in these patients. However, it should be considered that higher ultrafiltration volumes, that could lead to more effective reduction in left ventricular strain, could also induce hemodynamic instability and activation of adrenergic and renin-aldosterone systems, known stimulators of BNP production. So, the net effect of fluid removal during HD could vary according to the relative contribution of these factors. Interestingly, the 3 patients whose NT-proBNP levels increased after HD had high ultrafiltration volumes and high decrease in body weight and in proportional pulse pressure, favoring this hypothesis.

NT-proBNP RR was also not correlated to dialysis efficacy index Kt/V; nevertheless, Kt/V is an index of clearance of urea, a small-molecular-weight molecule. NT-proBNP is an 8.5-kDa protein; consequently, its removal kinetics can be very different. HD duration was higher in patients with NT-proBNP RR above median and this can be an indirect marker of higher NT-proBNP removal.

Another factor that could have contributed to NT-proBNP reduction was residual renal function, which was not quantified in our sample. However, considering the duration of HD session and the degree of impairment of renal function, it is unlikely that a significant decrease would result from physiological NT-proBNP renal clearance during that period. Conceptually, residual renal function could have had more influence on NT-proBNP levels. An inverse relationship between NT-proBNP and 24-hour urine output in HD patients has been reported [8]. Whether the slight differences in clearance (or in other factors, like volume control) between HD patients with and without residual renal function would translate into significantly different NT-proBNP levels remains to be evaluated.

The net result of NT-proBNP variation during dialysis is probably dependent on the balance between factors interfering with NT-proBNP synthesis and elimination during a HD session, that remain, for the now being, largely speculative. The influence of dialysis on NT-proBNP and BNP concentrations needs to be more elucidated, even more if this HD-related modulation of the system seems to relate to prognosis.

The prognostic value of NT-proBNP has been extensively reported. It has been observed in all spectrum of heart failure severity and in ambulatory and acute setting. Despite this, limited information is available in patients with renal function impairment. Some studies have shown that NT-proBNP has prognostic value in patients with mild to moderate renal failure [5, 19, 20], but most studies on NP have excluded patients with moderate to severe renal failure, particularly HD patients.

In our sample, NT-proBNP proved to be a valuable prognostic marker of long-term hospitalization-free survival. There was a lack of significance in the association of pre-HD NT-proBNP levels with outcome, probably related to sample size, as can be intuited by analysis of Kaplan-Meier curves that clearly diverge between patients with levels above and below median. Post-HD NT-proBNP levels and NT-proBNP RR were associated with the outcome and only NT-proBNP RR remained significantly associated with prognosis on multivariate regression analysis, pointing to an increased robustness of these parameters compared to pre-HD levels. This finding is consistent with our previous studies on heart failure patients with and without renal failure that showed that the extent of treatment-induced NT-proBNP decrease, more than its absolute levels, is associated with prognosis [20, 21]. We and others also found that admission NT-proBNP levels in patients hospitalized due to acute heart failure are not good prognostic markers as they probably correspond to 'wet BNP' [20–22]; by analogy, pre-HD NT-proBNP can be considered 'wet BNP', and likewise was not predictive of prognosis in this study. This relationship between NT-proBNP reduction and event-free survival in our population argues in favor of a deactivation of the natriuretic peptides system during HD, similarly to that occurring during treatment of acute heart failure. We speculate that patients that have a bigger decrease in NT-proBNP levels following HD are those who suffer a real deactivation of natriuretic system, either by achieving effective decompression of LV pressure/volume overload and/or by not stimulating adrenergic and renin-aldosterone systems. Racek et al. [12] found a negative correlation between NT-proBNP change during HD and left ventricle ejection fraction, concluding that HD probably triggers production of NT-proBNP and that increase is emphasized by impaired LVEF. It is conceivable that patients that have subtle cardiac structural or functional abnormalities and/or suffer adrenergic or renin-aldosterone activation during HD, and hence show lower NT-proBNP reduction ratio and higher post-HD NT-proBNP levels (because of triggered production/secretion), are 'sicker' patients, more prone to the occurrence of adverse events.

Patients on HD have high rates of morbidity and mortality. Cardiovascular disease is a major contributor to this grim prognosis. In this group of patients without cardiac systolic dysfunction, NT-proBNP could predict hospitalization or death, independently of other known prognostic markers in this population, like LVMI, hemoglobin or albumin. This study suggests that serial quantification of NT-proBNP is helpful, not as a marker of fluid status, but in risk stratification of ESRD patients.

Limitations

Echocardiographic estimation of LVM has been widely used in HD patients but can be influenced by patients' volume status. We tried to minimize the effect of fluid changes in our patients by indexing LV mass to height$^{2.7}$ and by performing the exam in a non-HD day in all patients. Nevertheless, cardiac resonance imaging is the gold standard method for calculating LVM. Our cohort was of limited sample size and this fact may explain why other known prognostic indexes, like age and co-morbidities, did not reach significant prognostic impact. Only a few patients (n = 6) suffered cardiovascular events, leading to low statistical power in assessing these events. The low frequency of cardiovascular events is probably related to the absence of major cardiac functional abnormalities (namely left ventricle systolic dysfunction) in our sample. It also could be due to misclassification of some events.

Conclusions

NT-proBNP levels are increased in ESRD patients with normal systolic function, to the same magnitude whether they have left ventricular hypertrophy or not. NT-proBNP levels significantly decrease after high-flux HD session, apparently more as a consequence of dialysis clearance than of fluid removal. Post-HD levels and NT-proBNP decrease following HD are valuable long-term prognostic markers even in patients without systolic dysfunction. Further studies are required to fully understand the behavior of NP system in ESRD, including its HD-related variation and its relation with prognosis.

Acknowledgments

The authors acknowledge Dr. Rui Farinha for excellent technical assistance and Prof. Ana Azevedo for meaningful discussion.

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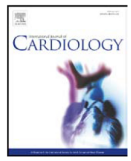
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IJCA-12215; No of Pages 6
International Journal of Cardiology xxx (2009) xxx–xxx



Contents lists available at ScienceDirect
International Journal of Cardiology
journal homepage: www.elsevier.com/locate/ijcard



BNP at discharge in acute heart failure patients: Is it all about volemia? A study using impedance cardiography to assess fluid and hemodynamic status☆

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ARTICLE INFO

Article history:
Received 9 March 2009
Received in revised form 4 June 2009
Accepted 11 August 2009
Available online xxxx

Keywords:
Hypervolemia
B-type natriuretic peptide
Predictors

ABSTRACT

Background: Besides hemodynamic parameters, several other variables have been associated to B-type natriuretic peptide (BNP) levels. Limited knowledge on BNP determinants in acute heart failure (HF) can undermine the interpretation of BNP levels.
Methods and results: To identify predictors of BNP levels, we evaluated 163 hospitalized acute HF patients. Thoracic fluid content (TFC) and hemodynamic parameters were measured by impedance cardiography at discharge. Patients were followed-up for 60 days for the occurrence of death/hospital admission. Median discharge BNP levels were 659.3 pg/ml. In multivariable linear regression analysis, TFC ($\beta = 0.043$, 95% CI 0.024–0.062 per U/kΩ, $p < 0.001$) was a powerful predictor of BNP levels, independently of known markers of HF severity like severe systolic dysfunction and discharge New York Heart Association class. Other independent predictors were: new onset HF, albumin, and body mass index. Sex, left cardiac work index, stroke index, hemoglobin, renal failure and discharge furosemide and lisinopril doses were associated to BNP only in univariate analysis. During follow-up, 45 (27.6%) patients were hospitalized or died. TFC (HR = 1.047 (1.016–1.080) per U/kΩ increase, $p = 0.003$) and BNP (HR = 1.003 (1.001–1.004) per 10 pg/ml increase, $p < 0.001$) were univariate predictors of the outcome, but in multivariate Cox regression analysis, only BNP was independently associated with prognosis.
Conclusion: Discharge BNP levels in acute HF patients reflected volemia and disease severity. Persistently high BNP levels during hospitalization should raise the possibility of remaining congestion, which could negatively influence prognosis. The utility of BNP as prognostic marker in HF may reside on its ability to reflect multiple underlying pathophysiological disturbances.
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1. Introduction

Natriuretic peptides, particularly B-type natriuretic peptide (BNP) and its amino-terminal portion, NT-proBNP, are now widely used as diagnostic and prognostic markers in heart failure (HF), in different clinical settings. In hospitalized acute HF patients, high BNP levels are associated with higher New York Heart Association (NYHA) classes and worse short and long-term event-free survival [1–6].

Nevertheless, the interpretation of BNP levels in acute HF may be challenging due to the number of potentially implicated contributors. In a recent analysis of previous studies, Balion et al. reported more than a hundred physiological, pathological, pharmacological, biochemical and hematological factors associated with BNP, although only a few with independent association (like age, female gender and creatinine concentrations) [7].

Ventricular stretch is considered the main stimulus for BNP secretion. Therefore, hypervolemia, causing ventricular volume and pressure overload, is conceptually the major contributor to the high BNP levels found during an episode of acute decompensation of HF. This assumption led to the concept of "wet BNP" proposed by Maisel [8] and is the rationale for evaluating and interpreting BNP levels at hospital admission and discharge.

There is, however, limited information on the relative influence of volemia, hemodynamic parameters and other less obvious factors like biochemical, hematological and clinical parameters on BNP levels in acute HF patients. The identification of potential confounders and their relative contribution to BNP levels is crucial in the interpretation

☆ This study was supported by a grant from Fundação de Ciência e Tecnologia (PIC/IC/82773/2007).
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doi:10.1016/j.ijcard.2009.08.001

Please cite this article as: Pimenta J, et al, BNP at discharge in acute heart failure patients: Is it all about volemia? A study using impedance cardiography to assess fluid and hemodynamic status, Int J Cardiol (2009), doi:10.1016/j.ijcard.2009.08.001

of that test and may have direct management implications, as it can argument for or against the need to intensify HF treatment or for a closer follow-up.

Impedance cardiography is a non-invasive method of measuring thoracic fluid content (TFC) and hemodynamic parameters using changes in thoracic electrical impedance, and it has been applied and compared with invasive hemodynamic measurements in HF patients [9,10].

To clarify the interpretation of BNP plasma concentration during an episode of acute HF, we aimed to identify impedance cardiography-derived, demographic, clinical, and laboratorial parameters independently associated with discharge BNP levels and to evaluate their relative importance. We also intended to characterize patients with persistently very high BNP levels (>1000 pg/ml) despite having received in-hospital treatment for acute HF. Additionally, we sought to evaluate the performance of impedance cardiography parameters in predicting short-term prognosis in acute heart failure patients, as compared to BNP levels.

2. Methods

2.1. Patients

We studied a convenience sample of 163 patients admitted to our department due to acute HF between October 2006 and October 2007, without acute coronary syndromes (defined as rise and/or fall of troponin I with at least one value above the 99th percentile of the upper reference limit, together with symptoms or ECG changes indicative of myocardial ischaemia), moderate-to-severe aortic valve disease or prosthetic aortic valves. Acute HF was defined as patients experiencing exacerbation of symptoms, with at least one NYHA class increase, or new diagnosis of HF. The diagnosis of HF was based on the European Society of Cardiology criteria [11].

2.2. Study procedures

Impedance cardiography was performed on the day of discharge with the patients in the supine position using a Niccomo Monitor (Cardiodynamics, San Diego, California) to measure TFC and hemodynamic parameters. This thoracic bioimpedance device uses two pairs of dual sensors (each consisting of two electrodes) placed 180° apart at the base of the neck and at the mid-axillary line at the level of xiphoid process. The outer electrodes of the sensors transmit a low-amplitude, high frequency current (2.5 mA, 70 kHZ) and the inner electrodes detect thoracic voltage changes. Changes in voltage are used to calculate changes in impedance. Baseline static thoracic impedance is indicative of chest fluid volume and the reciprocal of that parameter is termed thoracic fluid content (TFC). Dynamic impedance is affected by aortic blood volume and velocity and changes in thoracic impedance are processed to calculate stroke volume and cardiac output; other hemodynamic parameters are calculated using standard equations.

Blood samples were collected on discharge day. BNP was measured using a chemiluminescent immunoassay kit (Abbot AxSYM). Other biochemical analyses were performed at the hospital laboratory using standard methods. Estimated glomerular filtration rate (eGFR) was calculated using 4-variable Modification of Diet in Renal Disease (MDRD) equation [12,13] and renal failure was defined as eGFR < 60 ml/min/1.73 m^2.

Patients were classified as having preserved, mildly, moderately or severely depressed left ventricle systolic function according to qualitative assessment of left ventricle function on echocardiography.

Patients received standard treatment with diuretics (furosemide with or without spironolactone), angiotensin-converting enzyme (ACE) inhibitors and beta-blockers according to attending physicians. For study analysis we considered beta-blockers and ACE inhibitors dosage equivalence as follows: 10 mg of bisoprolol = 50 mg carvedilol and 150 mg captopril = 30 mg of lisinopril.

After discharge, patients were followed-up for 60 days for the occurrence of all-cause hospital readmission or death. Surveillance was made by direct contact with patients or relatives and observation of medical records.

The study was approved by the local Ethics Committee and patients gave informed consent.

2.2. Statistical analysis

We stored and analyzed data using SPSS (SPSS Inc, Chicago, Illinois). Unless otherwise noted, results are presented as median (interquartile range (IQR)) for quantitative variables and as counts and proportions (%) for categorical variables. Characteristics of patients with BNP > and ≤1000 pg/ml were compared using the Mann–Whitney test for continuous variables and the Chi-square test for categorical variables. Predictors of BNP levels were evaluated by linear regression analysis. BNP levels were logarithmized, achieving a normal distribution in the study sample. Univariate predictors were included in a multivariable model, built using a stepwise forward procedure. Standardized coefficients were used to directly compare the strength of the association of variables on different scales. *R*-squared was used to express the proportion of variability in BNP in the study sample that was explained by the model. Logistic regression was used to identify predictors of BNP > 1000 pg/ml. The associations were quantified using multivariable-adjusted odds ratios (OR) and 95% confidence interval (CI). The association of independent variables with time to outcome was assessed by Cox regression and is expressed as hazard ratio (HR) and 95% CI. In performing regression analysis, the relationship between the independent and dependent variables was evaluated and, whenever there was no linear association between them, independent continuous variables were dichotomized according to their median values. A significance level of 5% was used.

3. Results

Demographic and clinical characteristics of the 163 patients, 114 (69.9%) of male gender, median age 73.0 (61.0–80.0) years, are shown in Table 1. Systolic function was severely depressed in about half of the patients and 25.8% had preserved systolic function. Thirty-two patients (19.2%) had new onset HF.

Median BNP levels at discharge were 659.3 (253.0–1474.7) pg/ml, ranging from 47.0 to 10,323.2 pg/ml. Fifty-seven patients (35.0%) had discharge BNP levels >1000 pg/ml.

Results of univariate linear regression analysis for predictors of BNP levels at discharge are depicted in Table 2. Variables significantly associated to BNP levels in univariate analysis were included in the multivariate linear regression model (Table 3). TFC ($\beta = 0.043$, 95% CI 0.024–0.062 per U/kΩ, $p < 0.001$) and severe systolic dysfunction ($\beta = 0.831$, 95% CI 0.449–1.212, $p < 0.001$) were the most powerful predictors of BNP at discharge. New onset HF (versus decompensation of chronic HF), albumin and body mass index were negatively associated with BNP levels, while discharge NYHA class 3–4 was directly related to BNP levels. Sex, left cardiac work index, stroke index, hemoglobin, presence of renal failure and furosemide and lisinopril doses at discharge were excluded from the model. The R^2 value of the multivariate model was 47.6%.

Multivariate logistic analysis identified TFC, left ventricular systolic dysfunction, stroke index, new onset HF and albumin as predictors of BNP > 1000 pg/ml at discharge (Table 4).

Within 60 days of hospital discharge, 45 patients suffered the combined event of death or hospital readmission; 117 completed the follow-up period without any event and 1 patient could not be contacted after hospital discharge and was excluded from follow-up analysis. BNP was strongly related to prognosis as evaluated by univariate Cox regression analysis (Table 5). Of the parameters evaluated by impedance cardiography, only TFC was a univariate predictor of the outcome. Hemoglobin and albumin levels were also related to prognosis but in multivariate analysis only BNP and hemoglobin emerged as independent predictors of the outcome (Table 5).

4. Discussion

This study shows that volume status, as measured by TFC, is a major determinant of BNP levels in acute HF patients. Other factors like left ventricle systolic function, functional class and chronicity should be considered when interpreting BNP levels in that context.

Congestion is the most frequent challenge to deal with when treating HF. Unrecognized hypervolemia is frequently present in non-edematous HF patients and is associated with increased cardiac filling pressures and worse patient outcomes [14]. Approximately 50% of patients admitted for acute HF are discharged with persistent symptoms and/or minimal or no weight loss. The need to focus on a more accurate assessment and management of volume status in HF has been underscored [15,16]. In our sample, TFC was associated to the occurrence of hospital readmission or death within 2 months of discharge, supporting the importance of congestion as a predictor of worse prognosis.

Our results suggest that BNP measurement could be used as a marker of persisting congestion after in-hospital management of an

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Table 1
Characteristics of patients in the whole sample and according to BNP at discharge.

	Whole sample $n=163$	BNP ≤ 1000 pg/ml $n=106$	BNP > 1000 pg/ml $n=57$	p
Age (years)	73.00(61.00–80.00)	73.50(63.00–81.00)	71.00(58.50–78.50)	0.142
Male sex, *n* (%)	114(69.94)	69(65.09)	45(78.95)	0.066
New onset heart failure *n* (%)	32(19.63)	28(26.42)	4(7.02)	0.003
NYHA class >2 at discharge	25(15.34)	13(12.26)	7(12.28)	0.548
Body mass index (kg/m^2)	25.96(22.39–29.56)	26.60(22.55–30.47)	25.26(21.73–28.66)	0.119
BNP at admission (pg/ml)	1129.90(681.35–2094.50)	875.10(544.50–1318.60)	2334.30(1438.50–3738.80)	<0.001
BNP at discharge (pg/ml)	659.30(253.00–1474.00)	371.55(202.03–642.85)	2165.00(1297.80–3413.55)	<0.001
Hemoglobin at discharge, (g/dl)	12.30(10.70–14.20)	12.40(10.93–14.40)	12.10(10.40–13.75)	0.554
Creatinine at discharge (mg/dl)	1.15(0.95–1.52)	1.11(0.93–1.43)	1.29(1.00–1.67)	0.091
Renal failure at discharge, *n* (%)	80(49.10)	46(43.40)	34(59.6)	0.048
Atrial fibrillation, *n* (%)	78(47.9)	52(49.1)	26(45.6)	0.675
Echocardiographic LVSF				0.001
Normal, *n* (%)	42(25.8)	38(35.8)	4(7.0)	
Mildly depressed, *n* (%)	10(6.1)	6(5.7)	4(7.0)	
Moderately depressed, *n* (%)	30(18.4)	17(16.0)	13(22.8)	
Severely depressed, *n* (%)	81(49.7)	45(42.5)	36(63.2)	
Mean arterial pressure (mmHg)	77.00(69.00–85.00)	78.00(69.00–86.00)	74.00(69.00–83.00)	0.259
Heart rate (beats/min)	74.00(64.00–86.00)	73.00(62.75–83.00)	76.00(65.00–88.00)	0.181
Acceleration index (/s^2)	0.60(0.41–0.81)	0.61(0.44–0.84)	0.53(0.40–0.74)	0.166
Left cardiac work index (kg.m/m^2)	2.20(1.80–2.80)	2.20(1.90–2.80)	2.00(1.60–2.75)	0.066
Cardiac index (l/min/m^2)	2.15(1.85–2.60)	2.21(1.91–2.67)	2.06(1.70–2.42)	0.037
Stroke index (ml/m^2)	29.80(25.40–36.70)	31.30(26.83–37.10)	27.50(21.90–33.70)	0.004
Systolic time ratio	42.00(33.00–52.00)	41.00(33.00–48.25)	43.00(33.00–59.50)	0.162
Systemic vascular resistance index (dyne.s.cm^{-5}.m^2)	2718.00(2153.00–3290.00)	2636.50(2063.75–3261.50)	2891.00(2418.50–3377.00)	0.180
Thoracic fluid content (/kΩ)	32.60(28.00–37.10)	31.55(27.68–35.85)	34.90(29.50–44.75)	0.005
Dose of furosemide at discharge (mg/day)	80.00(40.00–120.00)	80.00(40.00–120.00)	120.00(80.00–160.00)	<0.001
Dose of lisinopril at discharge (mg/day)	10.00(5.00–10.00)	10.00(5.00–20.00)	5.00(2.50–10.00)	0.087
Dose of carvedilol at discharge (mg/day)	12.50(6.25–12.50)	12.50(6.25–12.50)	12.50(6.25–18.75)	0.858
Digoxin, *n* (%)	52 (31.90)	34 (32.10)	18 (31.60)	0.948
Spironolactone, *n* (%)	52 (31.90)	29 (27.40)	23 (40.40)	0.090

Continuous variables are expressed as median (interquartile range); NYHA — New York Heart Association; LVSF — left ventricle systolic function.

acute HF episode. TFC was associated to BNP levels, independently of markers of disease severity, like systolic dysfunction and NYHA class. Accordingly, discharge BNP levels may reflect concurrent mechanisms for persistent pulmonary congestion, such as excessive salt and water intake, renal failure contributing to hypervolemia or insufficient diuretic dose, other than known measures of HF severity. Worse symptoms of volume overload and increased cardiac filling pressures, reflected by higher NYHA classes at discharge, were also predictive of higher BNP levels. Altogether, these findings validate the theoretical concept of "wet BNP" in a clinical setting.

The assumption of BNP measurement as a method to refine volume assessment in acute HF can have direct therapeutic

Table 2
Univariate linear regression analysis for predictors of BNP levels at discharge (dependent variable: logarithmized BNP).

	Beta	95% CI	Standardized beta	p
Male gender (vs female)	0.712	0.345–1.079	0.289	<0.001
Age (per year)	0.000	−0.013–0.014	0.006	0.944
New onset heart failure (vs chronic decompensated)	−0.769	−1.195 to −0.343	−0.271	<0.001
Discharge NYHA class 3–4 (vs 1–2)	0.688	0.211–1.165	0.224	0.005
Left ventricle systolic function				
Preserved	0			
Mildly–moderately depressed (vs preserved)	0.757	0.284–1.229	0.288	0.002
Severely depressed (vs preserved)	0.826	0.419–1.233	0.366	<0.001
Thoracic fluid content (per U/kΩ)	0.049	0.029–0.069	0.358	<0.001
Systemic vascular resistance index (per dyne.s.cm^{-5}.m^2)	0.000	0.000–0.000	0.119	0.132
Stroke index (per ml/m^2)	−0.031	−0.051 to −0.011	−0.236	0.002
Left cardiac work index (per kg.m/m^2)	−0.285	−0.514 to −0.056	−0.190	0.015
Heart rate (per beat/min)	0.006	−0.005–0.018	0.085	0.283
Mean arterial pressure (per mm Hg)	−0.008	−0.022–0.006	−0.087	0.270
Systolic time ratio	0.010	−0.001–0.021	0.139	0.076
Acceleration index (/s^2)	−0.370	−0.945–0.205	−0.100	0.206
Velocity index (/1000/s)	−0.009	−0.019–0.000	−0.146	0.062
Hemoglobin (per g/dl)	−0.081	−0.158–0.003	−0.159	0.042
Serum sodium (per mEq/l)	−0.044	−0.089–0.001	−0.151	0.055
Serum albumin (per g/dl)	−0.792	−1.148 to −0.435	−0.326	<0.001
Renal failure at discharge	0.396	0.050–0.742	0.175	0.025
Body mass index (per kg/m^2)	−0.042	−0.076 to −0.007	−0.185	0.018
Atrial fibrillation	0.027	−0.325–0.379	0.012	0.879
Lisinopril dose > median[a]	−0.521	−0.886 to −0.156	−0.250	0.006
Furosemide dose > median[a]	0.720	0.369–1.071	0.309	<0.001
Carvedilol dose > median[a]	0.062	−0.438–0.562	0.024	0.806

NYHA — New York Heart Association.
[a] Median doses: lisinopril = 10 mg/day, furosemide = 80 mg/day, carvedilol = 12.5 mg/day.

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Table 3
Multivariate linear regression model for predictors of BNP levels at discharge (dependent variable: logarithmized BNP); $R^2 = 47.6\%$.

	Beta	95% confidence interval	Standardized beta	p
Thoracic fluid content (per U/kΩ)	0.043	0.024–0.062	0.341	<0.001
Left ventricle systolic function				
Preserved	0			
Mildly–moderately depressed (vs preserved)	0.579	0.150–1.009	0.242	0.009
Severely depressed (vs preserved)	0.831	0.449–1.212	0.392	<0.001
New onset heart failure (vs chronic decompensated)	−0.611	−0.965 to −0.256	−0.244	0.001
Serum albumin (per g/dl)	−0.533	−0.903 to −0.164	−0.225	0.005
Discharge NYHA class 3–4 (vs 1–2)	0.479	0.074–0.885	0.171	0.021
Body mass index (per kg/m^2)	−0.036	−0.067 to −0.005	−0.165	0.022

Excluded variables: Sex, left cardiac work index, stroke index, hemoglobin, renal failure, lisinopril dose at discharge, furosemide dose at discharge.
NYHA — New York Heart Association.

implications. Should diuretic dose be increased in acute HF patients with persisting very high BNP levels despite being adequately treated and lacking major clinical signs of hypervolemia? Or should therapy focus on optimizing doses of angiotensin-converting enzyme inhibitors and beta-blockers? It is probable that the "congestion-component" of BNP levels during an episode of acute HF is more important than in chronic HF, when BNP levels would reflect more global hemodynamic status (inotropism, peripheral vascular resistances). Consequently, diuretics could be more important in BNP-guided therapy during and in the period immediately following an acute HF episode than in ambulatory compensated HF patients.

The conflicting results of two previous studies evaluating BNP-guided therapy [17,18] may be related to this hypothesis. While in both STARS-BNP and STARBRITE studies patients in the BNP-guided arm received higher doses of ACE inhibitors and beta-blockers, only in STARS-BNP (the study with positive impact in event-free survival) they also received higher increments in diuretics. Moreover, STARBRITE patients were sicker patients randomized following a hospitalization for acute HF and target BNP was individualized according to BNP levels at discharge. As suggested by our results, discharge BNP levels can reflect persistent congestion and probably are not the best targets for maintaining patients free of HF-related events.

Although TFC was associated to short-term prognosis in univariate analysis in our sample, stressing the implication of congestion in event-free survival, its prognostic ability was lost when adjusted to BNP levels. BNP and hemoglobin levels at discharge were the only independent predictors of the outcome in our patients. Besides reflecting TFC, BNP levels in our patients were also related to other markers of heart failure severity (NYHA class, systolic function) and so, not surprisingly, BNP emerged as a better prognostic marker. These findings further support the idea that the excellent performance of BNP levels in predicting prognosis in heart failure relies on the capacity of natriuretic peptides system to mirror the multitude of pathophysiological alterations characteristic of the disease, such as hypervolemia, high cardiac filling pressures, myocardial distension, neuro-humoral activation, cardio-renal interactions. Natriuretic peptides system is modulated by each of those variables and its expression, being a reflection of all of them, is probably a better predictor of outcome than each one of the variables independently.

The inverse association of albumin to BNP levels that we have found could also reflect hypervolemia, as intravascular volume expansion can lead to hemodilution and decrease albumin concentration. On the other hand, patients with hypoalbuminemia can be more prone to pulmonary congestion, due to decreased colloid osmotic pressure [19]. Therefore, the link between BNP and serum albumin in HF patients could, at least partially, be explained by hypervolemia. Hypoalbuminemia may also be a reflection of advanced stages of the disease, when malnutrition, cachexia and inflammation could be implicated in a low albumin level [20–23]. A recent study reported considerably higher levels of BNP in patients with hypoalbuminemia versus patients with normal albumin levels in a large

Table 4
Multivariate logistic regression model for predictors of BNP levels >1000 pg/ml at discharge.

	Odds ratio	95% confidence interval	p
Thoracic fluid content (per U/kΩ)	1.054	1.004–1.106	0.032
Left ventricle systolic function[a] (preserved, mildly, moderately, severely depressed)	2.203	1.466–3.312	<0.001
New onset heart failure (vs chronic decompensated)	0.172	0.051–0.576	0.004
Stroke index (per ml/m^2)	0.938	0.888–0.992	0.024
Albumin (per g/dl)	0.391	0.158–0.965	0.042

Excluded variables: Systolic time ratio, renal failure and furosemide dose at discharge.
[a] Effect for linear trend.

Table 5
Cox regression model for predictors of hospital readmission/death within 60 days after discharge in acute heart failure patients.

	Hazard ratio	95% confidence interval	p
Univariate analysis			
Age (per year)	0.990	0.969–1.011	0.361
BNP at discharge (per 10 pg/ml)	1.003	1.001–1.004	<0.001
Hemoglobin (per g/dl)	0.750	0.646–0.871	<0.001
Albumin (>median[a])	0.503	0.273–0.927	0.027
Serum sodium (per mEq/l)	1.610	0.896–2.893	0.111
Renal failure	1.375	0.764–2.476	0.288
Discharge NYHA class 3–4	1.914	0.967–3.787	0.062
Moderate/severe systolic dysfunction	1.165	0.611–2.219	0.643
Thoracic fluid content (per U/kΩ)	1.047	1.016–1.080	0.003
Stroke index (>median[a])	0.774	0.430–1.393	0.393
Systemic vascular resistance index (>median[a])	1.403	0.776–2.534	0.262
Left cardiac work index (>median[a])	0.711	0.396–1.278	0.254
Systolic time ratio (>median[a])	1.102	0.614–1.980	0.744
Acceleration index (>median[a])	0.869	0.484–1.560	0.638
Velocity index (>median[a])	0.713	0.395–1.289	0.263
Multivariate analysis			
BNP at discharge (per 10 pg/ml)	1.002	1.001–1.004	<0.001
Hemoglobin (per g/dl)	1.757	0.648–0.885	<0.001

NYHA — New York Heart Association.
[a] Median values: albumin = 35.00(32.80–38.55) g/dl, Serum sodium = 138 (136–140) mEq/l, stroke index = 29.90(25.40–36.73) ml/m^2, Systemic vascular resistance index = 2719.00(2153.75–3303.25) dyne.s.cm^{-5}.m^2, Left cardiac work index = 2.20 (1.80–2.80) kg m/m^2, Systolic time ratio = 41.50(33.00–52.00), Acceleration index = 0.60 (0.41–0.81) /s^2, Velocity index = 33.70(23.53–47.15) /1000/s.

Please cite this article as: Pimenta J, et al, BNP at discharge in acute heart failure patients: Is it all about volemia? A study using impedance cardiography to assess fluid and hemodynamic status, Int J Cardiol (2009), doi:10.1016/j.ijcard.2009.08.001

ambulatory cohort with systolic HF; albumin was an independent marker of prognosis in that population [20].

Left ventricular systolic function assessed by echocardiography was an independent determinant of BNP levels. Several community and hospital-based studies have reported higher BNP levels in HF patients with systolic dysfunction as compared to patients with preserved systolic function [24–28]. The influence of cardiac contractile status on BNP levels was also reflected by the association between left cardiac work and stroke indexes with BNP in univariate analysis; nevertheless, only echocardiographic systolic function remained a significant predictor of BNP in the multivariate model. Whether the relationship between those impedance-derived hemodynamic variables and BNP would be stronger in compensated HF patients remains to be evaluated. Nevertheless, stroke index was an independent predictor of BNP levels >1000 pg/ml.

The natural history of HF seems to be important when interpreting BNP levels, as new onset HF was associated to lower BNP levels in our sample. Natriuretic peptides are a counter-regulatory response in HF and some degree of resistance to their action seems to develop as the disease progresses [29–31]. Additionally, hemodynamic profile deteriorates in end-stage HF, also stimulating BNP synthesis.

The inverse relationship between body mass index and BNP plasma concentration has been reported previously. While the pathophysiologic basis for this association is not understood, it seems that BNP retains its value as a marker for worse symptoms, impaired hemodynamics and prognosis in overweight and obese HF patients [32,33]. Nevertheless, interpretation of discharge BNP levels in obese/overweight patients may be more difficult and depend more on the knowledge of the patient's "dry" BNP than in lean patients.

Our model partially explained (by about 50%) BNP levels, raising the possibility that other, as yet unidentified, variables could influence those levels in vivo. Also, some variables previously reported to be associated with BNP (like hemoglobin, age, atrial fibrillation, renal failure or HF-related medications) were not significantly related to it or lost their association in multivariate analysis of our sample. These findings question the relevance of those parameters when interpreting BNP levels in acute HF patients.

4.1. Limitations

This observational study evaluates a convenience sample of hospitalized HF patients, with a high proportion of severe left ventricle systolic dysfunction. Our model partially explains BNP levels; other variables not included or evaluated in our sample may be associated with BNP levels. The assay used to measure BNP levels, like the other similar assays commercially available, could have detected other forms of BNP, besides mature BNP32 [34]; that possibility and its clinical relevance remain to be clarified.

4.2. Conclusions

Discharge BNP levels in acute HF patients reflect volemia as well as the severity of the underlying disease. Persistently high BNP levels during hospitalization due to acute HF should call to the attention for the possibility of persistent congestion, which can negatively influence prognosis. In that setting, the need to adjust diuretic therapy should be considered, particularly when physical signs of hypervolemia are absent. The interpretation of BNP levels in obese/overweight patients should take into consideration the lower levels of BNP in that population.

Acknowledgement

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [35].

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Please cite this article as: Pimenta J, et al, BNP at discharge in acute heart failure patients: Is it all about volemia? A study using impedance cardiography to assess fluid and hemodynamic status, Int J Cardiol (2009), doi:10.1016/j.ijcard.2009.08.001

Liver INTERNATIONAL
OFFICIAL JOURNAL OF THE INTERNATIONAL ASSOCIATION FOR THE STUDY OF THE LIVER

Liver International ISSN 1478-3223

CLINICAL STUDIES

B-type natriuretic peptide is related to cardiac function and prognosis in hospitalized patients with decompensated cirrhosis

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Keywords
B-type natriuretic peptide – cardiac output – impedance cardiography – cirrhosis – prognosis

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Received 25 January 2010
Accepted 6 April 2010

DOI:10.1111/j.1478-3231.2010.02266.x

Abstract

Background: B-type natriuretic peptide (BNP) concentrations are high in cirrhosis, possibly related to volume status and cirrhotic cardiomyopathy. The prognostic significance of BNP in cirrhosis is unknown. *Aims:* We aimed to evaluate (i) the influence of haemodynamic parameters and volaemia, assessed by impedance cardiography (ICG), in BNP levels, (ii) the performance of BNP as a prognostic marker, in a cohort of cirrhotic patients. *Methods:* Patients consecutively hospitalized with decompensated cirrhosis during 1 year were evaluated. At admission, ICG and BNP measurements were performed in 83 patients (median age 56 years; median Child–Pugh score = 10). The 70 patients discharged were followed for the occurrence of death within 6 months. *Results:* Median BNP levels were 130.3 (65.2–363.3) pg/ml. Independent BNP predictors in multivariate linear regression analysis were cardiac output, age and haemoglobin ($R^2 = 36.7\%$). The 24 patients with cardiac systolic dysfunction, defined by low cardiac output, had higher BNP concentrations than the other patients (230.8 vs 98.5 pg/ml, $P = 0.003$). BNP levels above median were associated with an increased occurrence of death within 6 months of discharge (log rank $P = 0.023$). Cardiac output and BNP were predictors of survival in univariate Cox regression analysis. Only BNP remained independently related to the outcome in multivariate analysis [hazard ratio = 2.86 (1.11–7.38), $P = 0.03$]. *Conclusions:* BNP levels in cirrhosis reflect cardiac systolic function and non-cardiac variables that should be considered in their interpretation. BNP is an independent predictor of medium-term survival in advanced cirrhosis, suggesting its utility in risk stratification of decompensated cirrhotic patients.

B-type natriuretic peptide (BNP) is a cardiac hormone secreted in response to ventricular volume and pressure overload. It has natriuretic, diuretic and vasodilator actions, regulating volume homeostasis. High BNP levels have been found in congestive states like heart and renal failure and chronic liver disease (1). In heart failure, BNP concentrations correlate with cardiac function and hypertrophy and appear to be proportional to congestion (the 'wet BNP') (2, 3). In the other conditions, the association with volume overload has not been established and may be confounded by the presence of intrinsic heart disease (uremic or cirrhotic cardiomyopathy).

Cirrhotic cardiomyopathy has been acknowledged as a condition of impaired cardiac contractility, systolic and diastolic dysfunction and electromechanical abnormalities in cirrhotic patients without known cardiac disease (4–6). Although increasingly accepted as a complication of liver failure, its clinical significance is still under debate, with a few reports suggesting an impact in prognosis (7–10).

The association between BNP concentrations and cardiac diastolic dysfunction in stable, ambulatory cirrhosis patients has been reported, suggesting that BNP could be a marker of cirrhotic cardiomyopathy (11–14). It remains uncertain whether BNP testing, now widely available, could be used to screen for cardiac dysfunction in routine clinical evaluation of chronic liver disease patients. The role of natriuretic peptides as prognostic markers in that setting has not been explored and remains speculative.

While invasive angiography is the gold standard for haemodynamic assessment in cirrhosis, its applicability is limited in the majority of clinical settings. Impedance cardiography (ICG) is a non-invasive, operator-

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independent method of measuring thoracic fluid content (TFC) and haemodynamic parameters using changes in thoracic electrical impedance, and it has been applied and compared with invasive haemodynamic measurements mainly in heart failure and critical care patients (15–17). Although there is still limited experience with the use of ICG in cirrhotic patients (18–21), a previous study showed good correlation between cardiac output and peripheral systemic resistance calculated by echocardiography and by ICG in patients with cirrhosis with and without ascites, concluding that ICG is a reliable method for assessing systemic haemodynamics in these patients (21).

To clarify the influence of cardiac function and volaemia, assessed by ICG, and other non-cardiac variables in BNP levels in cirrhotic patients we conducted a prospective study in hospitalized patients with decompensated cirrhosis. Additionally, we aimed to evaluate the performance of BNP as a medium-term prognostic marker in this setting. Our hypothesis was that BNP levels would be related to cardiac function and, by this means, to survival.

Patients and methods

We prospectively evaluated patients consecutively admitted to our department with the main diagnosis of decompensated cirrhosis (defined by the presence of ascites, encephalopathy, variceal bleeding and/or jaundice) during a 1-year period (July 2007–July 2008). The diagnosis of cirrhosis was based on clinical, laboratory and ultrasonographic criteria and was also confirmed by liver biopsy in 20.5% of the cases.

Clinical interview, physical examination, blood sample collection and ICG study were performed at admission.

A group of 13 healthy controls with similar age and sex distribution as the patient group was also submitted to clinical, analytical and ICG evaluation.

Impedance cardiography was executed with the patients in the supine position using a Niccomo Monitor (Cardiodynamics, San Diego, CA, USA) to measure haemodynamic parameters and TFC. This thoracic bioimpedance device uses two pairs of dual sensors (each consisting of two electrodes) placed 180° apart at the base of the neck and at the mid-axillary line at the level of xiphoid process. The outer electrodes of the sensors transmit a low-amplitude, high-frequency current (2.5 mA, 70 kHz) and the inner electrodes detect thoracic voltage changes. Changes in voltage are used to calculate changes in impedance. Baseline, static thoracic impedance is indicative of chest fluid volume, and the reciprocal of this parameter is termed TFC. Dynamic impedance is affected by aortic blood volume and velocity, and changes in thoracic impedance are processed to calculate stroke volume and cardiac output; other haemodynamic parameters are calculated using standard equations. Intra-individual coefficients of variation (CV) of cardiac output, systemic vascular resistance and TFC in our sample were calculated using three recorded consecutive measurements of those ICG parameters and were as follows: cardiac output CV = 3.3% (1.4–4.9%); systemic vascular resistance CV = 3.3% (1.3–5.0%) and TFC CV = 1.1% (0.5–1.8%).

B-type natriuretic peptide was measured using a chemiluminescent immunoassay kit (Abbott AxSYM, Abbott Laboratories, Abbott Park, IL, USA). The measurement range of this assay is 10–5000 pg/ml and it is designed to have an upper 95% confidence interval (CI) of 12% total coefficient of variation. Within-run and total % coefficient of variation according to BNP measurement are as follows: $\pm$ 180 pg/ml: < 6 and $< 7\%$; $\pm$ 1000 pg/ml: < 5 and $< 6\%$ and $\pm$ 3800 pg/ml: < 4 and $< 5\%$. Other biochemical analyses were performed using standard methods at the hospital laboratory. Estimated glomerular filtration rate (eGFR) was calculated using the four-variable modification of diet in renal disease equation (22) and renal failure was defined as eGFR < 60 ml/min/1.73 m^2.

Patients were followed for 6 months from discharge day. The primary endpoint was all-cause death. Surveillance was made by direct contact with patients or relatives and observation of medical records, by an investigator blinded to BNP levels.

This study was approved by the Institution Ethics Committee and patients or their relatives gave informed consent.

Statistical analysis

We stored and analysed data using SPSS (SPSS Inc., Chicago, IL, USA). Results are presented as median (25th–75th percentile) for quantitative variables and as n(%) for categorical variables. A significance level of 5% was used.

Predictors of BNP levels were evaluated by linear regression analysis. BNP levels were logarithmized, achieving a normal distribution in the study sample. Correlation coefficients were estimated using Pearson's correlation. Univariate predictors were included in a multivariable model, built using a stepwise forward procedure. Standardized coefficients were used to directly compare the strength of the association of variables on different scales. R^2 was used to express the proportion of variability in BNP in the study sample that was explained by the model. Differences in continuous variables between groups were compared by the use of Mann–Whitney test. The χ^2-test was use to compare proportions. Survival curves were estimated according to the Kaplan–Meier method and compared by the log-rank test. The association of independent variables with time to outcome was assessed by Cox regression and is expressed as hazard ratio (HR) and 95% CI. In performing regression analysis, the relationship between the independent and dependent variables was evaluated and whenever there was no linear association between them, independent continuous variables were dichotomized according to their median values.

Results

Ninety-one patients were admitted during the study period. Of those, eight had a known history of heart

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failure and were excluded; they had significantly higher BNP levels than the rest of the patient sample [869.1 (597.9–1414.4) vs 130.3 (65.2–363.3) pg/ml, $P < 0.001$]. Eighty-three patients, age 56.0 (47.0–70.0) years, 51 (61.4%) of the male gender were evaluated in the subsequent analysis. Clinical, laboratorial and haemodynamic parameters of the patients and controls are shown in Table 1. Fourteen patients (16.9%) were medicated with β-blockers in ambulatory – their BNP levels did not differ from the rest of the sample (153.9 vs 120.1 pg/ml, $P = 0.429$). Eight patients had medium/large volume pleural effusion – they had higher TFC (44.1 vs 35.3 1/k Ω, $P = 0.021$) but similar BNP concentrations compared with the other patients (104.4 vs 133.2 pg/ml, $P = 0.723$).

Determinants of B-type natriuretic peptide

The associations between LnBNP levels and demographic characteristics, medications, aetiology and staging of chronic liver disease, physical exam parameters, laboratorial analysis and ICG-derived parameters were evaluated using univariate linear regression analysis (Table 2). The variables significantly associated with LnBNP were included in a multivariable linear model, displayed in Table 3. Age, cardiac output and haemoglobin levels were independent predictors of LnBNP levels. R^2 for the model was 36.7%.

B-type natriuretic peptide and cardiac function

The median values of ICG-derived haemodynamic parameters in the patient sample were within the normal range (Table 1).

Cardiac output ($r = -0.38$, $P < 0.001$) and systemic vascular resistance ($r = 0.29$, $P = 0.008$) were correlated to LnBNP. TFC was directly and significantly related to LnBNP ($r = 0.28$, $P = 0.009$). There was no correlation between LnBNP and systolic time intervals: left ventricle ejection time ($r = 0.16$, $P = 0.141$), systolic time ratio ($r = -0.05$, $P = 0.685$) and pre-ejection period ($r = 0.02$, $P = 0.889$).

Table 1. Demographical, clinical, laboratorial and haemodynamic characteristics of the patients and controls

	Patients	Controls	
Characteristics	*n* = 83	*n* = 13	*P*
Female gender (*n*, %)	32 (38.4)	4 (30.8%)	0.540
Age (years)	56 (47–70)	55 (51–62.5)	0.953
Aetiology			
Alcoholic (*n*, %)	62 (74.7)		
Viric (*n*, %)	14 (16.9)		
Other (*n*, %)	7 (8.4)		
Child–Pugh Class:			
A (*n*, %)	1 (1.2)		
B (*n*, %)	30 (36.1)		
C (*n*, %)	52 (62.7)		
Child score (points)	10 (9–12)		
Physical exam			
Ascites (*n*, %)	57 (68.7)	0 (0)	< 0.001
Peripheral oedema (*n*, %)	51 (61.4)	0 (0)	< 0.001
Pulmonary rales (*n*, %)	14 (16.9)	0 (0)	0.109
Jugular venous distension (*n*, %)	23 (27.7)	0 (0)	0.030
Blood analysis			
Haemoglobin (g/dl)	11.2 (9.8–12.8)	14.6 (14.0–15.8)	< 0.001
Platelet count ($\times 10^9$/L)	87 (55–131)	238 (214–300)	< 0.001
BNP (pg/ml)	130.3 (65.2–363.0)	24.7 (10.0–38.0)	< 0.001
Sodium (mEq/L)	135 (131–139)	139 (138–140)	0.002
Albumin (g/dl)	2.56 (2.08–2.89)	4.19 (4.04–4.46)	< 0.001
Total bilirubin (mg/dl)	3.33 (2.01–6.63)	0.52 (0.44–0.60)	< 0.001
Creatinine (mg/dl)	0.95 (0.73–1.20)	1.09 (0.87–1.13)	0.389
Prothrombin time (sec)	17.7 (15.5–20.7)	11.9 (11.4–12.7)	< 0.001
Haemodynamic parameters			
Heart rate (beats/min)	87 (77–97)	71 (68–83)	0.008
Mean arterial pressure (mmHg)	85 (76–95)	86 (77.5–94)	0.864
Cardiac output (L/min)	5.9 (4.3–7.3)	6.3 (5.6–7.0)	0.383
Systemic vascular resistance (dyne/s/cm^5)	1074 (908–1564)	1016 (802–1252)	0.222
Velocity index (1/1000/s)	56.2 (41.6–71.0)	55.0 (30.5–69.5)	0.514
Pre-ejection period (ms)	83 (67–96)	92 (79–106)	0.082
Left ventricle ejection time (ms)	273 (238– 304)	292 (280–324)	0.078
Systolic time ratio	32 (24–39)	31 (28–36)	0.752
Thoracic fluid content (1/k Ω)	36.0 (30.2–43.6)	27.2 (23.8–28.7)	< 0.001

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Table 2. Univariate linear regression analysis for predictors of B-type natriuretic peptide levels at admission (dependent variable: logarithmized B-type natriuretic peptide)

	Beta	95% confidence interval	*r*	*P*
Female gender (vs male)	− 0.358	− 0.834–0.117	− 0.164	0.138
Age (per year)	0.036	0.021–0.050	0.467	< 0.001
Alcoholic aetiology (vs other)	− 0.102	− 0.641–0.437	− 0.042	0.707
Large/medium volume ascites (vs small/none)	− 0.008	− 0.483–0.467	− 0.004	0.975
Peripheral oedema	0.263	− 0.215–0.742	0.121	0.277
Pulmonary rales	0.763	0.160–1.366	0.269	0.014
Jugular venous distension	0.326	− 0.194–0.854	0.137	0.216
Moderate/large pleural effusion	− 0.066	− 0.861–0.729	0.018	0.869
Child score > 10	0.095	− 0.376–0.566	0.045	0.689
Serum sodium (per mEq/L)	0.049	0.008–0.090	0.257	0.019
Serum albumin > 2.6 g/dl	− 0.515	− 0.97 to − 0.060	− 0.243	0.027
Haemoglobin (per g/dl)	− 0.146	− 0.253 to − 0.036	− 0.288	0.008
Renal failure*	0.777	0.281–1.272	0.328	0.003
Serum total bilirubin (per mg/dl)	− 0.025	− 0.062–0.012	− 0.148	0.183
Prothrombin time (per second)	− 0.011	− 0.052–0.030	− 0.058	0.605
Platelet count (per 1×10^9/L)	− 0.002	− 0.005–0.001	− 0.134	0.229
Heart rate (per beat/min)	− 0.015	− 0.029 to − 0.001	− 0.231	0.036
Mean arterial pressure (per mmHg)	− 0.015	− 0.034–0.003	− 0.185	0.094
Cardiac output (per L/min)	− 0.189	− 0.292 to − 0.086	− 0.376	< 0.001
Thoracic fluid content (per U/kΩ)	0.026	0.007–0.045	0.283	0.009
Systemic vascular resistance (per dyne/s/cm^5)	0.001	0.000–0.001	0.293	0.008
Pre-ejection period (per ms)	0.001	− 0.009–0.010	0.016	0.889
Left ventricle ejection time (per ms)	0.004	− 0.001–0.008	0.163	0.141
Systolic time ratio	− 0.004	− 0.026–0.017	− 0.045	0.685
Velocity index (1/1000/s)	− 0.010	− 0.019 to − 0.001	− 0.246	0.025
Previous spironolactone use	0.275	− 0.191–0.741	0.129	0.244
Previous furosemide use	0.295	− 0.172–0.761	0.138	0.212
Previous β-blocker use	0.190	− 0.435–0.815	0.067	0.546

*Renal failure defined as eGFR < 60 ml/min/1.73 m^2.

Table 3. Multivariate linear regression model for predictors of B-type natriuretic peptide (BNP) levels at admission (dependent variable: logarithmized BNP); $R^2 = 37.6\%$

	Beta	95% confidence interval	Standardized β	*P*
Age (per year)	0.024	0.009–0.040	0.323	0.003
Cardiac output (per L/min)	− 0.159	− 0.272 to − 0.047	− 0.298	0.006
Haemoglobin (per g/dl)	− 0.148	− 0.240 to − 0.057	− 0.297	0.002

Excluded variables: Heart rate, velocity index, systemic vascular resistance, thoracic fluid content, pulmonary rales, renal failure, albumin > 2.6 g/dl, sodium.

B-type natriuretic peptide levels were significantly higher in the 24 patients with systolic dysfunction, defined by a cardiac output below the lower normal range (< 4.5 l/min) compared with the other patients with a cardiac output above this cutoff [230.8 (121.8–502.5) vs 98.5 (50.1–197.2) pg/ml, $P = 0.003$] (Fig. 1). Median TFC values were similar in patients with low and in patients with normal/high cardiac output (38.3 vs 35.3 1/ kΩ, $P = 0.48$). The proportion of patients with low cardiac output was similar in the group with alcoholic cirrhosis and in the group with other aetiologies (29.0 vs 28.6%, $P = 0.968$); BNP levels did not differ between the two groups [123.1 (63.2–368.0) vs 132.7 (76.2–293.6), $P = 0.737$] respectively.

Five patients had cardiac output above and systemic vascular resistance below the normal range – these patients with a hyperdynamic circulation pattern had BNP levels that did not differ from those of the rest of the sample. The 12 patients who had both measurements of cardiac output in the fourth quartile and systemic vascular resistance in the first quartile had significantly lower BNP levels [76.6 (36.8–121.7) vs 138.4 (71.3–377.4), $P = 0.017$].

B-type natriuretic peptide and prognosis

The 13 patients who died during the index hospitalization had higher BNP levels [213.0 (92.7–553.6) pg/ml] than the patients who survived [122.9 (56.1–277.3) pg/ml], although this difference did not reach statistical significance ($P = 0.069$).

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During the follow-up period, 21 patients died (six of haemorrhage, four of porto-systemic encephalopathy, eight of infections, two of non-liver cancer and one of sudden death) and three were lost to follow-up. BNP levels dichotomized according to the median (130.3 pg/ml) were associated with the occurrence of all-cause death at 6 months of follow-up, as evaluated by Kaplan–Meier analysis (log rank test $P = 0.023$) (Fig. 2). In univariate Cox regression analysis, BNP levels and cardiac output were significantly related to the outcome, but in multivariate analysis only BNP levels emerged as independent predictors of survival at 6 months (Table 4).

Discussion

Our results show that in decompensated cirrhosis patients: (i) BNP levels are related to cardiac systolic function as well as to other non-cardiac factors (age and haemoglobin levels) and (ii) BNP levels are associated with medium-term survival, suggesting its potential utility as a prognostic marker in cirrhosis.

Several studies have documented high natriuretic peptide levels in chronic liver disease, essentially in more advanced disease, and both in patients with and without alcoholic aetiology (11–16). The analysis of our sample

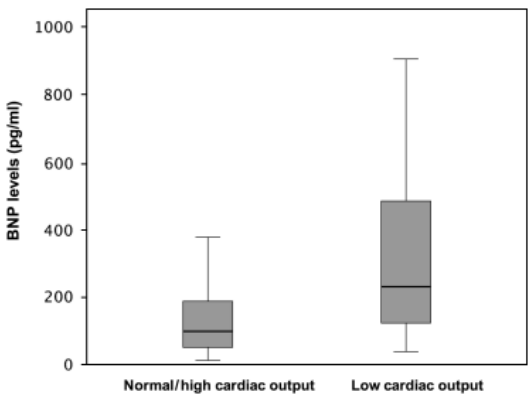


Fig. 1. Plasma B-type natriuretic peptide levels of patients with normal/high cardiac output and of patients with low cardiac output (98.5 pg/ml vs 230.8 pg/ml, $P = 0.003$); error bars represent 95% confidence interval).

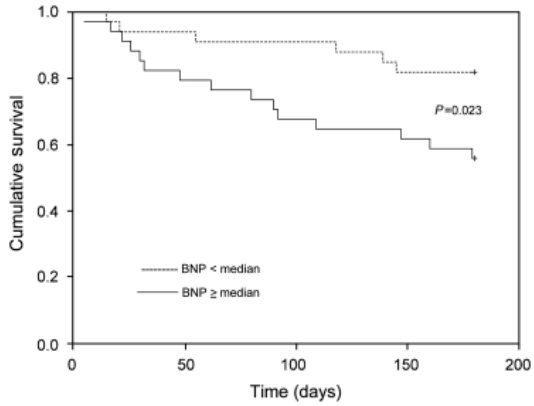


Fig. 2. Cumulative 6-months survival after hospitalization because of decompensated cirrhosis in patients with admission BNP levels above and below the median (Median BNP levels = 130.3 pg/ml; log rank test $P = 0.023$).

Table 4. Cox regression model for predictors of all-cause death within 6 months after discharge in cirrhosis patients

	Hazard ratio	95% confidence interval	*P*
Univariate analysis			
Age > 55 years	2.40	0.97–5.95	0.059
BNP > 130.3 pg/ml	2.86	1.11–7.38	0.030
Haemoglobin > 11.6 g/dl	1.45	0.61–3.45	0.396
Albumin > 2.6 g/dl	1.10	0.47–2.58	0.832
Serum sodium > 136 mEq/L	0.93	0.40–2.20	0.874
Creatinine clearance < 60 ml/min/1.73 m^2	0.95	0.35–2.59	0.919
Total bilirubin > 2.9 g/dl	1.20	0.50–2.89	0.689
Prothrombin time > 17.4 s	1.28	0.54–3.04	0.575
Child–Pugh score > 10 points	1.19	0.49–2.86	0.706
MELD score > 16 points	1.52	0.63–3.67	0.353
Thoracic fluid content > 35.3 U/kΩ	1.12	0.43–2.91	0.813
Cardiac output > 5.8 L/min	0.40	0.16–0.99	0.048
Systemic vascular resistance > 1087 dyne/s/cm^5	1.86	0.77–4.48	0.169
Pre-ejection period > 82	0.82	0.35–1.92	0.642
Left ventricle ejection time > 278	0.71	0.30–1.68	0.430
Systolic time ratio > 31	1.42	0.60–3.38	0.424
Velocity index > 54.5/1000/s	0.68	0.29–1.61	0.377
Multivariate analysis			
BNP > 130.3 pg/ml	2.86	1.11–7.38	0.030

BNP, B-type natriuretic peptide; MELD, model for end-stage liver disease.

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extends those reports and shows that in cirrhotic patients with a known history of heart failure, BNP levels are considerably higher than in those patients with no diagnosis of heart failure. These latter had high BNP levels but to a much lower magnitude than that usually expressed in heart failure patients.

Cardiac systolic function, evaluated by ICG-measured cardiac output, emerged as one of the independent predictors of BNP levels in our sample. The 24 patients (28.9%) who had cardiac output measurements below the normal range, indicating systolic function impairment, had significantly higher BNP levels than the rest of the patient sample. These observations suggest that natriuretic peptide system is activated in response to cardiac systolic incompetence in cirrhosis patients.

Impaired cardiac contractility with systolic dysfunction has been acknowledged as a feature of cirrhotic cardiomyopathy (4–6), possibly preceded by a state of isolated diastolic dysfunction. Evidence of impaired systolic function in cirrhotic patients without known heart failure has been reported recently. Decreased cardiac output has been implicated in the development of hepatorenal syndrome (10) and Krag and colleagues have found a relation between the low cardiac index evaluated by myocardial scintigraphy and an increased risk of progression to hepato-renal syndrome and death (23). Using a different method of haemodynamic assessment, we also found a subset of patients with a low cardiac output and confirmed the impact of cardiac output on prognosis.

Systolic dysfunction can be clinically silent and may only be apparent as a blunted response to cardiovascular stresses such as exercise, volume challenge or infections (4–6). It is conceivable that some of those stresses, particularly infections, fluid infusions and transfusions, were present in our acutely ill patients, contributing to lower cardiac output. A potential contribution of hypovolaemia, as a determinant of preload, to low cardiac output also cannot be discarded, but the fact that TFC was similar in patients with and without low cardiac output argues against that possibility. While we cannot exclude the effect of alcohol consumption on the systolic function of our patients, the proportion of patients with cardiac output below normal in those with alcoholic cirrhosis was similar to the one found in the group with cirrhosis because of other aetiologies, as were BNP levels in these two groups.

Although initially proposed, a relation of causality between the activation of natriuretic peptide system and hyperdynamic circulation in cirrhosis patients has failed to be proved (12, 24). Our results are in line with those observations as patients who showed a hyperdynamic circulation pattern had significantly lower BNP levels than the rest of the sample. Moreover, the relation that we have found between cardiac output and systemic vascular resistance on one hand, and BNP levels on the other, was the opposite of what would be expected if natriuretic peptide secretion was because of hyperdynamism. These observations strongly support the idea that the activation of natriuretic peptide system in cirrhotic patients is not related to the hyperdynamic circulation by itself, but to the intrinsic cardiac dysfunction.

Contrasting with previous studies (12, 13), we did not find an association between BNP levels and Child–Pugh score, a relation that has been explained by a progression of cardiac impairment with increasingly worse liver failure. The majority of our patients had severe chronic liver disease (52.7% in Child class C; median Child–Pugh score of 10 points), and that homogeneity might have contributed to reveal parameters more directly related to BNP concentrations than such a non-specific marker of cardiac dysfunction like Child–Pugh score.

B-type natriuretic peptide was also associated with central volaemia, as evaluated by TFC, and with the presence of pulmonary rales but not with other markers of congestion, like ascites and peripheral oedema. Considering that BNP is secreted in response to ventricular volume overload, those results were not surprising, and again, reinforce the utility of BNP as a marker of cardiac strain in liver failure patients. Besides volaemia, ICG determined TFC might also reflect the presence of pleural effusion, a possible complication of cirrhosis. While patients with moderate/large volume pleural effusion had higher TFC, their BNP levels did not differ from the other patients, nor the presence of pleural effusion was a predictor of BNP levels in our sample.

Other factors beyond cardiac function appear to influence BNP concentrations and thus should be considered when interpreting them. Age and haemoglobin were independent predictors of BNP levels in our patients, in a way similar to what has been consistently reported in the general population and heart failure and non-heart failure individuals (25–29). While the exact mechanism underlying the relation between BNP and haemoglobin levels is still to be fully elucidated, it may be related to volume overload and the consequent haemodilution.

Decompensated cirrhosis is a condition with grim prognosis. While death in cirrhotic patients generally occurs because of end-stage liver failure or portal hypertension, the hypothesis that cirrhotic cardiomyopathy could influence the clinical outcomes of those patients has been raised recently (7–10).

Our results support a role for cirrhotic cardiomyopathy as a determinant of survival, as lower cardiac output and higher BNP levels emerged as predictors of 6-month death in univariate analysis. Nevertheless, only BNP was independently associated with the outcome, reflecting the interaction between cardiac output and BNP levels. This finding was not unexpected, considering that BNP is a known marker of cardiac systolic function, as has been confirmed in our sample. However, as was also the case with our patients, BNP levels can be influenced by other haemodynamic variables, as well as non-cardiac-related parameters, like age, renal function, haemoglobin and even albumin, a marker of liver failure. Importantly, BNP appears to reflect cardio-renal

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interaction, more than simply cardiac dysfunction (30). With all this in mind, it appears plausible that BNP levels, a mirror of all this milieu, might be of prognostic usefulness in cirrhotic patients, whose survival greatly depends on the degree of liver failure and renal function, besides possibly on cardiac function.

This is, to the best of our knowledge, the first prospective study assessing the utility of BNP in prognostic stratification in cirrhosis. Although it suggests a promising role for BNP in that context, it should be regarded as exploratory, needing confirmation from further studies. Supporting our findings, a recent study has reported higher mortality in advanced cirrhosis patients with cardiac systolic dysfunction, a finding that failed to be predicted by the model for end-stage liver disease score (23).

Limitations

This is a single centre study performed in a heterogeneous sample of decompensated cirrhosis patients, with severe liver failure mainly because of alcohol consumption and submitted to acute therapeutic interventions. However, addressing a 'real-world' population, similar to every day clinical practice patients, may render our results more applicable to this setting. Impedance cardiography, while being reported as a reproducible mean of measuring cardiac output with good agreement with echocardiography in cirrhosis, is not the gold standard for haemodynamic assessment in that condition. TFC generally reflects central volaemia but may also be high in patients with pleural effusion.

Conclusions

B-type natriuretic peptide levels in cirrhosis patients mirror cardiac function and non-cardiac factors like age and haemoglobin, which should be considered when interpreting BNP measurements. BNP levels were independently associated with medium-term survival in decompensated cirrhotic patients, possibly as a reflection of cardiac systolic dysfunction. The role of natriuretic peptides as prognostic predictors in cirrhosis should be further explored, as they appear to add important information on top of classical markers of prognosis.

Acknowledgements

This study was supported by a grant from Hospital de S. João.

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V. Discussão

5.1. O SISTEMA DOS PN ESTÁ ACTIVADO NOS ESTADOS DE HIPERVOLÉMIA

Nos estudos por nós desenvolvidos, foi consistente o achado de níveis plasmáticos elevados de BNP/NT-próBNP nos insuficientes cardíacos, nos insuficientes renais e também nos cirróticos, embora com magnitudes diferentes, sendo a sua expressão menor nestes últimos. Ainda que a activação do sistema dos PN nestas patologias que têm em comum um estado de expansão de volume seja consensual, porque apoiada num número robusto de observações, as causas a ela subjacentes permanecem por elucidar.

Insuficiência renal

Tal como se depreende das análises dos estudos 1 e 2, os insuficientes renais parecem ter níveis significativamente elevados de NT-próBNP, principalmente os doentes com insuficiência renal moderada/grave ou terminal. Esta activação dos PN na insuficiência renal crónica terminal foi encontrada mesmo em doentes sem disfunção ventricular sistólica. Documentámos também uma diminuição significativa das concentrações de NT-próBNP em resposta à terapêutica da insuficiência cardíaca aguda e à hemodiálise.

A associação entre disfunção renal e concentrações aumentadas de BNP/NT-próBNP poderá resultar, pelo menos parcialmente, da dependência do rim para a eliminação e/ou excreção desses PN. Apesar de classicamente ter sido descrita uma maior dependência da eliminação renal no caso das moléculas de NT-próBNP[1-3], uma vez que, ao contrário do BNP, não sofre degradação activa pelos receptores NPR-C e pela NEP, um estudo recente aponta para um grau de extracção renal similar das duas moléculas de PN, da ordem dos 16-22%[4], sugerindo que estes dois marcadores apresentam equivalente dependência da função renal para a sua eliminação. Assim, a perda de função renal justificará em parte os níveis elevados de PN nos insuficientes renais. O facto de nos nossos

doentes com insuficiência cardíaca e insuficiência renal, os níveis de NT-próBNP serem progressivamente superiores nas classes de disfunção renal mais avançada, suporta este conceito. Permanecem, no entanto, por esclarecer o aumento progressivo da *ratio* NT-próBNP/BNP plasmática nos insuficientes renais, bem como outros mecanismos de degradação/eliminação do NT-próBNP[5].

É sobejamente conhecida a prevalência aumentada de disfunção cardiovascular nos insuficientes renais, constituindo uma das principais causas de morbilidade e mortalidade nestes indivíduos[6-8]. Os doentes com disfunção renal têm prevalência aumentada de doença coronária, hipertrofia ventricular e disfunção ventricular diastólica e sistólica, o que poderá também contribuir para a activação do sistema dos PN. No estudo 2, não conseguimos encontrar uma associação entre massa ventricular e níveis plasmáticos de NT-próBNP em doentes em hemodiálise, possivelmente por falta de poder estatístico para demonstrar esta associação. Outros estudos contemporâneos do nosso descrevem essa associação[9-11] bem como a relação com disfunção ventricular[12,13]. No entanto, o grau de activação do sistema dos PN na insuficiência renal poderá dificultar o estabelecimento de limiares diagnósticos para os níveis de NT-próBNP nessa circunstâncias. Deste modo, a utilidade diagnóstica do NT-próBNP na insuficiência renal, pelo menos em estádios avançados, poderá ser mais limitada.

Considerando que um dos estímulos mais potentes para a secreção dos PN é a sobrecarga de volume a nível ventricular, seria expectável que marcadores de volume na insuficiência renal se relacionassem com as concentrações plasmáticas de BNP/NT-próBNP e com a sua variação durante uma sessão de hemodiálise com ultrafiltração. No entanto, nos nossos doentes com insuficiência renal terminal não identificámos uma associação entre marcadores de volume, como o aumento de peso interdialítico, a perda de peso consequente à hemodiálise e o volume de ultrafiltração, e os valores plasmáticos de NT-próBNP ou a sua variação com a hemodiálise. Outros estudos que abordaram este assunto tiveram resultados díspares[13-16]. No presente momento não há evidência robusta que permita utilizar os PN como marcadores de peso seco nos doente em hemodiálise[14,17]. O nosso estudo 2 também contribuiu para a noção cada vez mais consubstanciada de que os níveis de BNP/NT-próBNP não são meros marca-

dores de volémia na IR terminal. No entanto, verificámos uma descida significativa das concentrações plasmáticas destes biomarcadores em resposta à hemodiálise (no caso da insuficiência renal crónica terminal – estudo 2) ou à terapêutica da insuficiência cardíaca aguda (no caso dos insuficientes renais com insuficiência cardíaca aguda – estudo 1). Esta constatação apoia a possibilidade de que alguma dessa redução nos níveis de BNP/NT-próBNP se deva ao tratamento da hipervolémia.

As concentrações de BNP/NT-próBNP poderão também relacionar-se com a activação neurohumoral (nomeadamente dos sistemas adrenérgico e renina-angiotensina-aldosterona) subjacente aos doentes com insuficiência cardíaca e insuficiência renal. Como já vimos, a síntese de BNP pode ser estimulada pelos sistemas adrenérgico e renina-angiotensina-aldosterona. A resposta do BNP/NT-próBNP à terapêutica da insuficiência renal terminal e da insuficiência cardíaca que encontrámos poderá ser também ser reflexo da modulação neurohumoral que essa terapêutica condiciona. Por um lado, a melhoria da sobrecarga de pressão e volume cardíacos e a consequente melhoria do perfil hemodinâmico poderão causar uma desactivação dos sistemas neuro-humorais. Por outro lado, nas circunstâncias em que as variações hemodinâmicas condicionadas pela hemodiálise e pela terapêutica diurética e vasodilatadora conduzam a hipotensão e hipovolémia poderá ocorrer estimulação adicional dos sistemas adrenérgico e renina-angiotensina-aldosterona que, por sua vez, induzirão síntese de BNP.

A proporção de doentes com uma descida significativa (≥ 30%) dos níveis de NT-próBNP em resposta à terapêutica da insuficiência cardíaca aguda foi semelhante em todas as classes de taxa de filtração glomerular que analisámos no estudo 1. Isto sugere que pelo menos uma parte dos níveis de NT-próBNP nos doentes com insuficiência renal é passível de ser reduzida pelo tratamento deplector de volume e manipulação neurohumoral.

A descida das concentrações de NT-próBNP em resposta à hemodiálise que verificámos nos doentes do estudo 2 poderá também explicar-se pelos mesmos motivos – no entanto, como já vimos, não encontrámos relação entre marcadores de variação de volume e descida de NT-próBNP. Adicionalmente, parece ocorrer remoção activa do NT-próBNP pela diálise de alto fluxo, ao contrário do que sucede na de baixo fluxo[15,18]

e a maior duração da sessão de hemodiálise nos nossos doentes que apresentaram reduções mais importantes do NT-próBNP pode relacionar-se com este facto.

Insuficiência cardíaca

Como seria de esperar, os doentes com insuficiência cardíaca aguda avaliados nos estudos 1 e 3 apresentavam níveis plasmáticos de BNP/NT-próBNP substancialmente elevados, que diminuíram após terapêutica. Não obstante, uma proporção significativa (35%) dos doentes manteve níveis de muito elevados de BNP (>1000 pg/ml) após o tratamento hospitalar.

No que se refere aos doentes com insuficiência cardíaca aguda, os níveis plasmáticos de BNP/NT-próBNP parecem ser um bom indicador do estado de volémia. No estudo 3, o conteúdo de fluído torácico, determinado por cardiografia de impedância eléctrica e reflexo do volume central, foi um dos preditores major dos níveis de BNP à alta. Este parâmetro também se associou a níveis pré-alta de BNP>1000 pg/ml. Esta associação do BNP com o fluído torácico foi independente de marcadores de gravidade da doença como o grau de disfunção sistólica ou a classe funcional da "New York Heart Association". Assim, e tendo em linha de conta os nossos resultados, a persistência de valores elevados de BNP após terapêutica da insuficiência cardíaca aguda deve fazer suspeitar da presença de congestão significativa, ainda que não clinicamente evidente. Sabe-se que a presença de hipervolémia clinicamente indetectável é frequente nos doentes com insuficiência cardíaca e associa-se a pior prognóstico[19,20]. Concentrações elevadas de BNP na pré-alta hospitalar devem, por isso, levar a considerar um ajuste na terapêutica diurética ou a presença de factores que contribuam para a hipervolémia como o excesso de ingestão hidro-salina ou a insuficiência renal.

O efeito da disfunção ventricular sistólica como preditor dos níveis de BNP documentado nos nossos doentes corrobora múltiplos estudos prévios[21-25]. De igual modo, está também bem estabelecida a associação que encontrámos entre níveis de BNP e classe funcional da "New York Heart Association"[26,27]. Já o facto dos nossos doentes com insuficiência cardíaca *de novo* apresentarem níveis significativamente mais baixos de BNP do que os doentes com insuficência cardíaca crónica descompensada não foi descrito

previamente (tanto quanto é do nosso conhecimento), embora se enquadre na fisiopatologia e história natural da doença. Os PN são uma resposta contra-regulatória aos mecanismos retentores de sódio e água e vasopressores progressivamente activados na insuficiência cardíaca à medida que esta se agrava. Existe também um certo grau de resistência aos PN na insuficiência cardíaca mais avançada[28-30]. Por outro lado, há evidência recente de que os níveis plasmáticos de BNP/NT-próBNP detectados pelos ensaios comerciais disponíveis (e utilizados nos nossos estudos) poderão corresponder não exclusivamente à forma madura de BNP1-32, mas a uma mistura heterogénea de peptídeos, alguns dos quais sem actividade biológica[31-33]. Isto parece ser mais importante nos doentes com insuficiência cardíaca avançada[33].

Para além dos factores directamente relacionados com a insuficiência cardíaca, encontrámos uma associação independente entre os níveis de BNP e o índice de massa corporal e a concentração plasmática de albumina. A influência de marcadores não-cardíacos nos níveis de BNP poderá condicionar a sua interpretação num contexto clínico. Numa análise recente, foram encontrados mais de cem factores associados ao BNP, embora apenas alguns de forma independente[34].

Tal como nos nossos doentes com insuficiência cardíaca, a relação inversa entre índice de massa corporal e os níveis plasmáticos de BNP foi descrita noutros estudos em doentes com e sem insuficiência cardíaca[35-39]. Apesar disso, o valor prognóstico dos PN parece conservado nos doentes obesos[35,37]. A interpretação dos valores plasmáticos de BNP/NT-próBNP nos doentes obesos poderá, portanto, ser mais difícil, principalmente se não fôr conhecido o nível de BNP basal (“BNP seco”).

A associação que encontrámos entre BNP e albumina plasmáticos poderá ter várias explicações. Por um lado, a hipervolémia poderá causar hemodiluição e, consequentemente, níveis mais baixos de albumina. A diminuição da pressão osmótica colóide também poderá facilitar o desenvolvimento de congestão pulmonar[40]. Por outro lado, a hipoalbuminemia pode ser um marcador de insuficiência cardíaca avançada, associando-se a desnutrição, caquexia e inflamação[41-44].

Em suma, a expressão de BNP/NT-próBNP está activada de forma exuberante na insuficiência cardíaca aguda. Os valores plasmáticos desses PN reflectem essencial-

mente a volémia e a gravidade da patologia subjacente. Verifica-se uma desactivação do sistema dos PN em resposta à terapêutica da insuficiência cardíaca aguda.

Cirrose hepática

No estudo 4 pretendemos estudar o sistema dos PN na cirrose hepática descompensada. Documentámos níveis de BNP elevados nos nossos doentes, corroborando estudos anteriores[45-49]. Os valores de BNP desses doentes eram substancialmente inferiores aos dos nossos doentes dos estudos 1 a 3, sugerindo que o sistema dos PN está activado na cirrose mas num grau inferior ao verificado na insuficiência cardíaca e renal. No entanto, essa expressão aumentada parece dever-se efectivamente ao aumento da síntese dos PN, já que a sua extracção hepática está preservada[47]. Apesar da disfunção renal se associar a níveis mais elevados de BNP na análise univariada da nossa amostra, essa associação perdeu significado na análise multivariada, sugerindo que a eliminação renal também não será um factor major no aumento do BNP na cirrose descompensada.

Assim, o aumento da síntese de BNP nestas circunstâncias parece dever-se à presença de disfunção cardíaca, tal como indicam os nossos resultados. Encontrámos uma associação independente entre BNP e débito cardíaco medido por cardiografia de impedância torácica. Verificámos também que o grupo de doentes com valores de débito cardíaco inferiores aos normais de referência para o método (4,5 l/min) apresentava níveis significativamente mais altos de BNP. Apesar alguns de trabalhos prévios apontarem para a associação dos níveis de PN com marcadores de função diastólica[46,48,49] e de alterações electrofisiológicas cardíacas[47], sugerindo a sua relação com a presença de cardiomiopatia cirrótica, a associação com parâmetros de disfunção sistólica não tinha sido descrita, tanto quanto é do nosso conhecimento. No entanto, a incompetência sistólica é reconhecida como um dos marcadores de cardiomiopatia cirrótica, ainda que a disfunção diastólica e as alterações electrofisiológicas pareçam ser mais frequentes[50-52]. A presença de disfunção sistólica e o seu impacto no prognóstico em doentes com cirrose avançada foi documentada recentemente por outros autores[53,54]. Considerando os resultados do nosso estudo, o BNP poderá ser um bom marcador de disfunção sistólica e, portanto, de cardiomiopatia cirrótica em doentes com cirrose descompensada.

Encontrámos uma associação entre a volémia central (expressa pelo conteúdo de fluído torácico determinado por cardiografia de impedância torácica) e os níveis de BNP dos nossos doentes, que não se manteve quando ajustada para outros parâmetros. Uma possível relação dos PN com a sobrecarga de volume que ocorre na cirrose tinha já sido sugerida pela associação dos níveis de BNP com o volume ventricular telediastólico[46] e com o volume plasmático e sanguíneo[47]. No entanto, a ausência de significado na análise multivariada aponta para que essa associação seja dependente de outros factores (como *p.e.* da presença de disfunção sistólica).

Nos doentes com cirrose descompensada, o valor de hemoglobina foi um preditor independente do BNP. A presença de anemia associa-se a concentrações de BNP mais elevadas em doentes com e sem insuficiência cardíaca[55,56]. Também na cirrose um estudo prévio relatou correlação inversa entre BNP e hemoglobina[48]. O elo causal desta associação permanece por esclarecer, embora possa relacionar-se com a sobrecarga de volume e consequente hemodiluição ou com a hipóxia e estimulação neuro-humoral provocadas pela anemia.

A idade é, segundo o nosso estudo, um factor a ter em conta na interpretação dos valores de BNP na cirrose. A correlação directa e independente da idade com os valores plasmáticos de BNP na nossa amostra assemelha-se ao que tem sido descrito na população geral e também em doentes com insuficiência cardíaca[57,58].

Da análise dos nossos resultados, podemos concluir que o sistema dos PN se encontra activado na cirrose descompensada, em relação com a presença de disfunção cardíaca. O BNP poderá ser um marcador de cardiomiopatia cirrótica nesse contexto. As concentrações plasmáticas de BNP devem, no entanto, ser interpretadas considerando a idade e o valor de hemoglobina destes doentes.

5.2. OS PN BNP E NT-PRÓBNP SÃO PREDITORES DE PROGNÓSTICO ROBUSTOS NOS ESTADOS DE HIPERVOLÉMIA

Insuficiência cardíaca

O valor prognóstico destes biomarcadores na insuficiência cardíaca está hoje amplamente demonstrado. Também nos nossos estudos 1 e 3 foi documentada esta excelente acuidade prognóstica. Os valores de NT-próBNP à alta e principalmente a descida significativa (≥ 30%) da sua concentração durante o internamento por insuficiência cardíaca aguda associaram-se à sobrevida livre de internamento aos 6 meses após a alta hospitalar, independentemente de outros marcadores de prognóstico como a função sistólica ventricular, classe da "New York Heart Association", idade, sexo, hemoglobina, sódio sérico, tensão arterial sistólica e frequência cardíaca. No estudo 1 comprovámos, assim, o melhor prognóstico associado a uma efectiva desactivação do sistema dos PN em resposta à terapêutica dirigida à descompensação da insuficiência cardíaca, tal como foi descrito anteriormente por nós e outros autores[59,60].

No estudo 3 avaliámos preditores de sobrevida livre de internamento aos 2 meses e verificámos que o BNP à data da alta, o conteúdo de fluído torácico, a hemoglobina e albumina se associavam significativamente ao evento em análise univariada em insuficientes cardíacos. Só o BNP e a hemoglobina se mantiveram como preditores independentes de prognóstico em análise multivariada, traduzindo a associação entre níveis plasmáticos de BNP e a volémia e albuminémia que já tínhamos descrito.

Assim, o BNP e o NT-próBNP são excelentes marcadores de prognóstico na insuficiência cardíaca aguda, mesmo em doentes com insuficiência renal, o que provavelmente se deve à capacidade do sistema dos PN de reflectir várias vertentes implicadas na fisiopatologia e na progressão dessa doença: sobrecarga de volume, estado hemodinâmico, activação neuro-humoral, interacção coração-rim.

Insuficiência renal

Como já vimos, os níveis de NT-próBNP foram preditores independentes de prognóstico nos doentes com insuficiência cardíaca aguda e insuficiência renal concomitantes

do estudo 1. Os resultados deste estudo têm maior relevância se considerarmos que se questionava a utilização do NT-próBNP em doentes com insuficiência renal significativa, dada a possível maior dependência da função renal para a sua eliminação, relativamente ao BNP. Demonstrámos, portanto, que a utilidade prognóstica a médio prazo do NT-próBNP se mantém mesmo em insuficientes cardíacos com disfunção renal ligeira-moderada, o que só estava estabelecido para prognóstico a curto prazo em doentes no serviço de urgência[61,62]. Além disso, pudemos ainda verificar que também nos doentes com insuficiência renal, mesmo em estádios mais avançados, há desactivação do sistema dos PN, que esta ocorre numa proporção de doentes semelhante à dos indivíduos com insuficiência cardíaca sem disfunção renal e que essa resposta tem implicações prognósticas.

De igual modo, as concentrações plasmáticas de NT-próBNP associaram-se ao prognóstico na coorte de insuficientes renais terminais em hemodiálise analisada no estudo 2. Os valores de NT-próBNP pós-hemodiálise e principalmente o grau de descida de NT-próBNP associada à sessão de hemodiálise foram preditores de morte/readmissão hospitalar subsequente (este último de forma independente quando ajustado para outras variáveis com significado prognóstico); as concentrações de NT-próBNP pré-hemodiálise não se associaram significativamente ao evento. Estes resultados sugerem que também nestes doentes uma resposta do sistema dos PN à terapêutica de redução da volémia se associa a melhor prognóstico a longo prazo. Apontam também para uma maior relevância em termos de prognóstico dos níveis de BNP “seco”, em consonância com o estudo 1 e com trabalhos prévios em insuficientes cardíacos[59,63].

Cirrose hepática

Nos últimos anos tem-se assistido a um interesse crescente no estudo das alterações cardíacas associadas à cirrose, que constituem a entidade denominada cardiomiopatia cirrótica. Este interesse foi reforçado pela constatação de que a disfunção cardíaca parece influenciar o prognóstico dos doentes com cirrose[53,54,64-66]. Apesar disso, o valor prognóstico do BNP/NT-próBNP na cirrose permanecia desconhecido.

No estudo 4, propusemo-nos a determinar a associação dos níveis plasmáticos de BNP e de variáveis hemodinâmicas avaliadas por cardiografia de impedância com a sobrevida aos 6 meses de uma coorte de doentes hospitalizados com cirrose descompensada.

Verificámos que o débito cardíaco e os níveis de BNP dicotomizados de acordo com a sua mediana se associaram significativamente à ocorrência de morte em análise univariada. No entanto, apenas o BNP emergiu como variável independente de prognóstico na análise multivariada, o que poderá ser explicado pelo facto do BNP ser um marcador de débito cardíaco baixo nesta amostra, tal como em muitos outros contextos. Na nossa amostra, o BNP foi melhor preditor de prognóstico do que marcadores de prognóstico clássicos como as pontuações de Child-Pugh e MELD, possivelmente devido à homogeneidade da distribuição destes últimos no nosso grupo de indivíduos com cirrose descompensada e agudamente doentes.

A relação do BNP com o prognóstico em doentes com cirrose avançada poderá então ser explicada pela sua associação com a presença de cardiomiopatia cirrótica mas possivelmente também por outros factores não-cardíacos (como a idade, a função renal, o valor de hemoglobina e de albumina) influenciarem a sua concentração plasmática. O facto do BNP ser um bom marcador do eixo cardio-renal poderá ser significativo, já que a disfunção renal influencia de forma relevante a sobrevida dos doentes com cirrose.

Apesar deste estudo ser, tanto quanto é do nosso conhecimento, o primeiro estudo prospectivo a avaliar a acuidade prognóstica do BNP na cirrose e necessitar, por isso, de confirmação, aponta para possibilidade da utilização este biomarcador na estratificação de risco de doentes com cirrose avançada.

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VI. Conclusões

- O sistema dos PN e, especificamente, a expressão dos biomarcadores BNP e NT-próBNP encontram-se activados nos estados de hipervolémia – insuficiência cardíaca, insuficiência renal e cirrose hepática.
- O aumento do BNP é proporcional ao grau de hipervolémia na insuficiência cardíaca. Na cirrose e na insuficiência renal não encontrámos uma associação independente entre volume e concentrações séricas de BNP/NT-próBNP.
- A concentração de BNP é determinada pela função cardíaca na insuficiência cardíaca e na cirrose. Não se identificou uma relação entre massa ventricular e níveis de NT-próBNP em doentes com insuficiência renal em hemodiálise.
- Nas amostras estudadas, o BNP e NT-próBNP associaram-se de forma independente e significativa ao prognóstico, sugerindo a possibilidade da aplicação destes biomarcadores na estratificação de risco não só na insuficiência cardíaca (onde o seu uso já está estabelecido), mas também na cirrose hepática e insuficiência renal.

VII. Resumo/Abstract

RESUMO

O sistema dos PN desempenha um papel fundamental na regulação da homeostasia hidroelectrolítica, sendo um dos mecanismos responsáveis pela promoção da diurese e natriurese. Desse modo, foi colocada a hipótese de existir deficiência dessas hormonas nos estados de hipervolémia, caracterizados por retenção inapropriada de sódio e água. No entanto, vários estudos demonstraram um aumento das concentrações dos PN nessas circunstâncias, embora as causas desse fenómeno estejam por esclarecer cabalmente.

Os estudos que compõem esta dissertação pretenderam esclarecer a expressão dos PN (nomeadamente do BNP e NT-próBNP) em 3 patologias associadas a hipervolémia: insuficiência cardíaca, insuficiência renal e cirrose hepática, e avaliar as implicações clínicas dela resultantes.

Num grupo de doentes com insuficiência cardíaca aguda e com diferentes estádios de insuficiência renal, verificámos que existia um aumento das concentrações de NT-próBNP proporcional à gravidade de disfunção renal mas que a resposta do sistema dos PN à terapêutica aguda estava preservada e tinha importante valor prognóstico. Numa outra amostra de doentes com insuficiência cardíaca aguda encontrámos uma associação independente entre a concentração plasmática de BNP e marcadores de volémia e disfunção cardíaca, realçando a importância desta hormona como marcador de congestão e de deterioração hemodinâmica. Já em insuficientes renais terminais documentámos uma descida significativa das concentrações de NT-próBNP em resposta a uma sessão de hemodiálise mas essa variação não se correlacionou com marcadores de volémia. Também em indivíduos com cirrose hepática descompensada não se verificou uma associação independente dos níveis de BNP com um marcador de volémia (conteúdo de fluído torácico determinado por

bioimpedância), embora esses parâmetros apresentassem uma correlação directa significativa na análise univariada. Nesses doentes, o BNP associou-se a disfunção cardíaca sistólica e poderá ser um marcador de cardiomiopatia cirrótica.

Considerando que os mais plausíveis determinantes dos valores de BNP/NT-próBNP nas patologias estudadas nestes trabalhos – disfunção hemodinâmica e congestão – poderiam ter impacto significativo no prognóstico, colocámos a hipótese dos níveis dessas hormonas serem bons marcadores de sobrevida nesse contexto. Verificámos que os valores de NT-próBNP pré-alta hospitalar e a sua diminuição em resposta ao tratamento se associaram à sobrevida livre de internamento em doentes com insuficiência cardíaca aguda com e sem disfunção renal. De igual modo, os níveis de BNP foram um dos mais robustos marcadores de prognóstico a curto prazo nos doentes com insuficiência cardíaca aguda. Na insuficiência renal crónica terminal, o valor de NT-próBNP pós-hemodiálise e principalmente o grau de descida desse biomarcador em resposta à hemodiálise associaram-se de forma independente ao prognóstico, sobrepondo-se a outros marcadores clássicos de sobrevida nesses doentes. A mesma capacidade discriminativa de prognóstico foi encontrada na investigação que realizámos em doentes com cirrose hepática. Comprovámos uma clara associação dos níveis de BNP com a ocorrência de morte aos 6 meses após a alta hospitalar, independente de outros factores reconhecidamente implicados no desfecho dessa patologia.

Assim, podemos concluir que há uma activação do sistema dos PN nos 3 estados de hipervolémia estudados. Na insuficiência cardíaca aguda a volémia parece ser um determinante major das concentrações de BNP/NT-próBNP, enquanto que na cirrose hepática e na insuficiência renal essa associação não é tão manifesta. A disfunção hemodinâmica desempenha também um papel importante na activação do sistema dos PN na insuficiência cardíaca aguda e na cirrose hepática descompensada. O BNP e o NT-próBNP são bons marcadores de prognóstico na insuficiência cardíaca mas também na insuficiência renal crónica terminal e na cirrose hepática. Esta boa acuidade prognóstica poderá advir do facto destes biomarcadores reflectirem um conjunto de variáveis como a função cardíaca/hemodinâmica, o grau de congestão, a função renal, a activação neuro-humoral e ainda outros parâmetros como a presença de anemia ou

de hipoalbuminemia e a idade, que reconhecidamente influenciam o prognóstico dessas patologias.

Os nossos estudos sugerem o interesse na determinação dos PN na insuficiência cardíaca (onde já está bem estabelecido) mas também na insuficiência renal crónica terminal e na cirrose hepática, já que poderá ser um método complementar de avaliação e estratificação de risco.

ABSTRACT

Natriuretic peptides play a major role in the control of body fluid homeostasis, promoting diuresis and natriuresis. Accordingly, it was postulated that natriuretic peptide deficiency would be found in hypervolemic states, characterized by inappropriate water and salt retention. However, several studies have reported high natriuretic peptides levels in that context, although the underlying causes have not been fully elucidated.

The studies compiled in this thesis aimed to evaluate the expression of natriuretic peptides system (namely BNP and NT-proBNP) in 3 hypervolemic states: cardiac failure, chronic kidney disease and liver cirrhosis.

In a sample of acute heart failure patients with different degrees of kidney failure, we have found high NT-proBNP levels that were proportional to the severity of renal dysfunction. Nevertheless, the response of NT-proBNP levels to treatment was similar, irrespective of renal function impairment, and the extent of that response had important prognostic value. In another group of acute heart failure patients we documented an independent association between BNP levels and markers of volemia and cardiac dysfunction, stressing the utility of BNP as a marker of congestion and hemodynamic deterioration in that setting. On the other hand, patients with end-stage renal disease had high NT-proBNP levels that significantly decreased after a hemodialysis session, but that reduction was not correlated with markers of volemia. We also could not find an independent association between BNP levels and thoracic fluid content, a surrogate of central volemia determined by bioimpedance, in patients with decompensated cirrhosis, although these parameters were significantly correlated in univariate analysis. In those patients BNP was associated to cardiac systolic dysfunction, suggesting that it could be a marker of cirrhotic cardiomyopathy.

Considering that in the conditions that we were studying the more plausible determinants of BNP/NT-proBNP levels would be hemodynamic dysfunction and congestion, we have formulated the hypothesis that those hormones would be related to prognosis. Therefore we intended to evaluate the prognostic utility of BNP/NT-proBNP in the 3 hypervolemic states. Pre-discharge NT-proBNP levels and its decrease during hospitalization were significantly related to the occurrence of death/hospital readmission in acute cardiac failure patients. Likewise, BNP emerged as one robust independent prognostic parameter in acute heart failure. In chronic kidney disease patients, post-hemodialysis NT-proBNP levels and, particularly, the extent of decrease in NT-proBNP levels following hemodialysis were independently related to the outcome, even when adjusted for classical prognostic markers in that setting. This good prognostic discriminative power was also found in patients with decompensated cirrhosis, in whom BNP levels were associated to 6-months survival, independently of known markers of prognosis.

In conclusion, natriuretic peptide system is activated in hypervolemic states: cardiac failure, chronic kidney disease and liver cirrhosis. In cardiac failure, volemia is a major determinant of BNP/NT-proBNP levels, while in chronic kidney disease and cirrhosis that relationship is less significant. Hemodynamic dysfunction plays an important role in the activation of natriuretic peptides system in acute heart failure and decompensated cirrhosis. BNP and NT-proBNP are good prognostic markers in acute heart failure but also in end-stage renal disease and in decompensated cirrhosis. This good prognostic acuity may result from the capacity of natriuretic peptides system to reflect several parameters like cardiac/hemodynamic dysfunction, congestion, renal function, neuro-humoral activation and other factors such as anemia, hypoalbuminemia or age, that can influence clinical outcomes in those conditions.

Our studies support the use of natriuretic peptides testing in the assessment and risk stratification of cardiac failure, chronic kidney disease and liver cirrhosis.