UNIVERSIDADE FEDERAL DE UBERLÂNDIA
INSTITUTO DE BIOTECNOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA E BIOQUÍMICA

Estabilidade de membrana de eritrócitos em pacientes com sepse, choque séptico e desordens hipertensivas da gestação

Alice Vieira da Costa

UBERLÂNDIA, MG
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Estudante: **Alice Vieira da Costa**
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Tese apresentada à Universidade Federal de Uberlândia como parte dos requisitos para obtenção do título de doutor em Genética e Bioquímica (área de Bioquímica)

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_____

Nilson Penha-Silva
(Orientador)

"Ora, àquele que é poderoso para fazer tudo muito mais abundantemente além daquilo que pedimos ou pensamos, segundo o poder que em nós opera."

(Efésios 3:20)

Dedico este trabalho...

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ABREVIATURAS

ATP	Adenosina Trifosfato
Crn	Creatinina
CRP	Proteína C Reativa, do inglês *C-Reactive Protein*
Hb	Hemoglobina
HDL-C	Colesterol da Lipoproteína de Alta Densidade
Ht	Hematócrito
LaBFiQ	Laboratório de Biofisicoquímica da Universidade Federal de Uberlândia
LDL-C	Colesterol da Lipoproteína de Baixa Densidade
MCH	Hemoglobina Corpuscular Média, do inglês *Mean Corpuscular Hemoglobin*
MCHC	Concentração de Hemoglobina Corpuscular Média, do inglês *Mean Corpuscular Hemoglobin Concentration*
MCV	Volume Corpuscular Médio, do inglês *Mean Corpuscular Volume*
PE	Pré-Eclâmpsia
RBC	Células Vermelhas do Sangue, do inglês *Red Blood Cells*
RDW	Variação da Distribuição de Volume de Células Vermelhas, do inglês *Red-Cell Distribution Width*
ROS	Espécies Reativas de Oxigênio
SIRS	Síndrome da Resposta Inflamatória Sistêmica
SOFA	*Sequential Organ Failure Assessment*
SUS	Sistema Único de Saúde
t-C	Colesterol Total
TGC	Triglicérides
UFU	Universidade Federal de Uberlândia
UTI	Unidade de Terapia Intensiva
VLDL-C	Colesterol da Lipoproteína de Muito Baixa Densidade

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APRESENTAÇÃO

O Laboratório de Biofisicoquímica (LaBFiQ) do Instituto de Biotecnologia da UFU tem-se dedicado desde o início deste século ao estudo do comportamento físicoquímico de eritrócitos, com o foco na influência de solutos (Cunha, Arvelos et al. 2007, Penha-Silva, Arvelos et al. 2008, Fonseca, Arvelos et al. 2010), na alimentação (Custodio Afonso Rocha, Ramos de Arvelos et al. 2012, de Arvelos, Rocha et al. 2013), atividade física (Paraiso, de Freitas et al. 2014, Paraiso, Goncalves et al. 2017), envelhecimento (Penha-Silva, Firmino et al. 2007, de Freitas, Marquez-Bernardes et al. 2013) e diversos processos patológicos (de Freitas, de Oliveira et al. 2010, Mascarenhas Netto, Fabbri et al. 2014, Aires Rodrigues de Freitas, Vieira da Costa et al. 2018, Rodrigues, de Medeiros et al. 2018) na estabilidade de membrana dessas células. Estes estudos têm ajudado a entender, por exemplo, a influência da glicemia (Lemos, Marquez-Bernardes et al. 2011, Rodrigues, de Medeiros et al. 2018) e da lipidemia (Bernardino Neto, de Avelar et al. 2013), afetadas pela alimentação (de Arvelos, Rocha et al. 2013), atividade física (Paraiso, de Freitas et al. 2014, Paraiso, Goncalves et al. 2017) e processos patológicos – como obesidade e *diabetes mellitus* -, além da influência de fatores nutricionais específicos (Alves de Rezende, Coelho et al. 2009) – como ferro, folato e cobalamina - sobre variáveis específicas utilizadas na caracterização da estabilidade de eritrócitos. Estes estudos têm ajudado a entender a ampla e complexa rede de fatores que afetam o comportamento de eritrócitos através da eritropoiese e da influência de fatores presentes na circulação sanguínea, bem como as relações da estabilidade de eritrócitos com as variáveis hematimétricas. Um exemplo significante destas contribuições é a inserção do comportamento de eritrócitos na patofisiologia da aterosclerose e de suas consequências (Garrote-Filho, Bernardino-Neto et al. 2017).

Essa tese dá continuidade a esta tarefa, buscando investigar a rede de fatores inter-relacionados com a estabilidade de membrana de eritrócitos com duas diferentes condições patológicas, a sepse e algumas desordens hipertensivas da gestação.

A sepse é definida como uma disfunção de órgãos que envolve risco de vida, causada por uma resposta desregulada do hospedeiro à uma infecção. Embora grandes avanços no entendimento da sua patogenia e novas estratégias terapêuticas tenham surgido, sua prevalência e taxa de mortalidade permanecem elevadas. Os eritrócitos,

células que têm como principal função o transporte de oxigênio, sofrem alterações na sepse, como a redução da deformabilidade, o que dificulta o alcance dessas células a diversos tecidos, prejudicando a oxigenação e contribuindo para a falência múltipla de órgãos. A capacidade de deformabilidade dos eritrócitos é atribuída a características constitutivas, sendo uma delas a elasticidade da membrana plasmática. A membrana plasmática é fluida em decorrência de sua composição lipídica (fosfolipídios e colesterol) e pelo fato de estar ancorada a uma rede de proteínas do citoesqueleto. Alterações na composição da membrana podem fazer com que o eritrócito se torne mais rígido ou mais fluido, o que pode alterar a estabilidade osmótica destas células, tornando-as mais vulneráveis ou mais resistentes à lise. A avaliação da estabilidade osmótica do eritrócito na sepse, correlacionada com variáveis hematimétricas e bioquímicas sanguíneas pode permitir uma melhor compreensão dos fatores que afetam a estabilidade de eritrócitos nesta condição. Neste sentido, novos achados podem levar a um melhor entendimento dos mecanismos de alterações reológicas dos eritrócitos na sepse e seus efeitos no fluxo sanguíneo e transporte de oxigênio, o que pode ser importante no desenvolvimento de novas estratégias terapêuticas para melhorar a disponibilidade de oxigênio celular e, portanto, reduzir o risco de falência de órgãos.

As desordens hipertensivas da gestação ocorrem em quatro diferentes formas: pré-eclâmpsia/eclâmpsia, hipertensão crônica, pré-eclâmpsia sobreposta à hipertensão crônica e hipertensão gestacional. As relações da estabilidade de membrana de eritrócitos com parâmetros antropométricos, hemodinâmicos, hematológicos e bioquímicos na pré-eclâmpsia já foi alvo de estudos anteriores deste laboratório (Aires Rodrigues de Freitas, Vieira da Costa et al. 2018, Rodrigues, de Medeiros et al. 2018). O presente estudo busca comparar essas relações da estabilidade osmótica de eritrócitos em diferentes desordens hipertensivas da gestação.

De acordo com as normas até então vigentes do Programa de Pós-Graduação em Genética e Bioquímica da UFU, esta tese é constituída por uma fundamentação teórica (**Capítulo 1**) necessária para a compreensão dos capítulos seguintes, que discorrem sobre a influência da sepse e do choque séptico (**Capítulo 2**) e das desordens hipertensivas da gestação, como hipertensão gestacional, pré-eclâmpsia sobreposta à hipertensão crônica e pré-eclâmpsia grave (**Capítulo 3**), sobre a estabilidade de membrana de eritrócitos e as relações das variáveis de estabilidade de eritrócitos com parâmetros hematimétricos e lipidemia em voluntários recrutados no Hospital de Clínicas da UFU.

CAPÍTULO 1

Fundamentação Teórica

Eritrócitos: deformabilidade e estabilidade

Os eritrócitos são células que atuam no transporte de oxigênio para os tecidos, uma das suas principais funções. Na circulação sanguínea, os eritrócitos constantemente se deparam com situações em que sua forma precisa ser alterada. Uma dessas situações é a necessidade que essas células têm de atravessar capilares estreitos, cuja secção transversal pode ser de apenas um terço do diâmetro do próprio eritrócito (Mohandas, Clark et al. 1980, Chasis, Agre et al. 1988, Mohandas and Gallagher 2008).

A deformabilidade do eritrócito refere-se a sua habilidade de modificar sua forma em resposta a forças aplicadas, o que é um fator determinante no fluxo sanguíneo, especialmente para a microcirculação e transporte de oxigênio (Bujak, Wasilewski et al. 2015). Esta capacidade é atribuída a três características constitutivas: geometria celular, viscosidade citoplasmática e elasticidade da membrana plasmática (Evans, Mohandas et al. 1984).

A membrana plasmática de eritrócitos é composta por proteínas (52% em peso), lipídios (fosfolipídios e colesterol, 40%) e carboidratos (8%) e sua elasticidade depende da interação estrutural entre a membrana plasmática externa e o esqueleto proteico subjacente (Piagnerelli, Boudjeltia et al. 2003).

As proteínas de membrana têm um papel importante na manutenção da integridade física do eritrócito, garantindo assim a sua estabilidade (Liu and Palek 1980). Dentre as proteínas que estão relacionadas à estrutura e estabilidade ou que auxiliam na estabilização do citoesqueleto, destacam-se a proteína banda 3, proteínas transportadoras de íons e as glicoforinas A, B, C e D (Jarolim, Rubin et al. 1998, Murador and Deffune 2007). A porção intracelular que forma o citoesqueleto é constituída por espectrina, actina, proteína 4.1, proteína 4.2 e anquirina (Piagnerelli, Boudjeltia et al. 2003) (Figura 1). A espectrina é uma proteína considerada determinante na estabilidade, estrutura e forma do eritrócito (Naydenov and Ivanov 2011).

Outro fator que pode influenciar nas características de estabilidade e deformabilidade são as alterações na composição da membrana plasmática, que podem tornar o eritrócito mais ou menos rígido, alterando a estabilidade osmótica destas células, o que pode torná-las mais vulneráveis à lise. O aumento do teor de colesterol na membrana do eritrócito, por exemplo, causa uma diminuição da sua deformabilidade e fluidez, o que pode levar ao comprometimento das suas propriedades reológicas e da fragilidade osmótica (Ramanadham and Kaplay 1982, Kanakaraj and Singh 1989, Koter, Franiak et al. 2004). A principal fonte de colesterol para a membrana do

eritrócito é proveniente da interação dessas células com a LDL (Low Density Lipoprotein). Assim, um aumento na quantidade de LDL-C irá favorecer uma maior interação entre essa estrutura lipídica e os eritrócitos, com um consequente aumento da quantidade de colesterol na membrana dessas células. Um aumento de colesterol em relação aos fosfolipídios irá resultar em uma membrana plasmática mais rígida (Lange and Slayton 1982). Quanto maior a rigidez da membrana, menor é a deformabilidade do eritrócito, o que o torna mais vulnerável em situações em que precisa mudar de forma. Além disso, uma membrana demasiadamente rígida pode dificultar outros processos, como a difusão de oxigênio e outros nutrientes, seja diretamente ou por intermédio de canais proteicos. Portanto, o aumento da rigidez da membrana do eritrócito torna essas células menos funcionais (Chabanel, Flamm et al. 1983).

Obviamente, se a funcionalidade do eritrócito ficar comprometida, o transporte de oxigênio pode ser prejudicado. Isto deve ser levado em consideração na fisiopatologia de diversas doenças e condições clínicas, principalmente naquelas em que a hipóxia é um fator agravante, como é o caso da sepse – que afeta desde o transporte do oxigênio pelos eritrócitos até sua distribuição pela microcirculação e utilização final nas mitocôndrias (Bateman, Sharpe et al. 2017) – e das desordens hipertensivas na gestação – as quais se caracterizam por alterações circulatórias importantes, disfunção endotelial e hipóxia fetal (Mustafa, Ahmed et al. 2012, Shamshirsaz, Paidas et al. 2012, Vats and Paul 2016).

A sepse

A definição e conduta com relação à sepse vêm se modificando à medida que novos conhecimentos são adquiridos sobre essa síndrome. Em 1991, em uma conferência de consenso entre o *American College of Chest Physicians* e a *Society of Critical Care Medicine* foi proposto que a sepse resulta da "síndrome da resposta inflamatória sistêmica (SIRS)" frente a uma infecção, em que são observadas duas ou mais das seguintes manifestações clínicas: (1) uma temperatura corporal superior a 38
°C ou inferior a 36 °C; (2) frequência cardíaca maior que 90 batimentos por minuto; (3) taquipneia, manifestada por uma frequência respiratória superior a 20 respirações por minuto, ou hiperventilação, como indicado por uma $PaCO_2$ inferior a 32 mmHg; e (4) uma alteração na contagem de glóbulos brancos, que pode ser uma contagem superior a 12000/mm^3, uma contagem inferior a 4000/mm^3, ou a presença de mais de 10% de neutrófilos imaturos (Bone, Balk et al. 1992). Além disso, foram definidos outros

termos úteis na classificação da gravidade da sepse, tais como a "sepse severa" – quando há uma disfunção orgânica concomitante – e o "choque séptico" – caracterizado por uma hipotensão persistente induzida pela sepse apesar da reposição volêmica adequada (Bone, Balk et al. 1992). Mais tarde, no entanto, foi constatado que os critérios de SIRS estão presentes em muitos pacientes hospitalizados, incluindo aqueles que nunca desenvolveram uma infecção (Churpek, Zadravecz et al. 2015), tornando-se imprescindível a busca por novos parâmetros que pudessem assegurar um diagnóstico mais assertivo.

Não há ainda um teste padrão ouro para o diagnóstico da sepse, no entanto, adicionalmente às observações das manifestações clínicas, podem-se aliar observações dos parâmetros fisiológicos, bioquímicos, imunológicos e modificações em vias tais como cardiovascular, neuronal, autonômica, hormonal, bioenergética, metabólica e alterações nas vias de coagulação que são induzidas pela infecção (Singer, De Santis et al. 2004, Angus and van der Poll 2013, Hotchkiss, Monneret et al. 2013, Deutschman and Tracey 2014, Singer, Deutschman et al. 2016).

Atualmente, novos critérios clínicos e avaliação de alguns parâmetros laboratoriais foram adotados para identificação da sepse, sendo o conjunto chamado de escore SOFA (*Sequential Organ Failure Assessment*), que varia numa escala de zero a quatro, sendo que uma variação aguda de 2 pontos ou mais no SOFA pode ser associada à infecção e define o estado séptico (Singer, Deutschman et al. 2016). O escore SOFA engloba padrões clínicos de respiração, coagulação, cardiovasculares, do sistema nervoso central e de volume urinário, além de considerar dosagens de bilirrubina e creatinina. A sepse pode ainda evoluir para o choque séptico, situação na qual os pacientes apresentam nível de lactato maior que 2 mmol/L e sepse acompanhada de hipotensão persistente ainda que haja reposição volêmica adequada, necessitando de vasopressores para manter a pressão arterial média igual ou maior que 65 mmHg (Singer, Deutschman et al. 2016).

A sepse é então definida nos dias atuais como uma disfunção de órgãos que envolve risco de vida, causada por uma resposta desregulada do hospedeiro à uma infecção (Singer, Deutschman et al. 2016). Embora grandes avanços no entendimento da sua patogenia e novas estratégias terapêuticas tenham surgido, sua prevalência e taxa de mortalidade permanecem ainda elevadas (Pereira, Souza et al. 2007, Mayr, Yende et al. 2014, Shankar-Hari, Harrison et al. 2017).

Epidemiologia da sepse

A sepse pode estar relacionada a qualquer infecção, as mais comuns sendo aquelas associadas à pneumonia, infecção urinária, meningite e uso de cateteres, dentre outras. Uma infecção em qualquer parte do corpo pode evoluir para um quadro de sepse ou choque séptico (Kaukonen, Bailey et al. 2014).

Os casos de sepse nos hospitais do Brasil vêm aumentando gradativamente, como mostrou um estudo recente realizado por Quintano e colegas, que observaram um aumento de 50,5% na incidência de hospitalizações por sepse no SUS entre 2006 e 2015 (Quintano Neira, Hamacher et al. 2018).

Na UTI, a sepse e o choque séptico são os quadros que mais matam. Cerca de 54% dos pacientes em UTI tem sepse e a mortalidade chega a 60%. Outro problema importante é a permanência elevada desses pacientes em UTIs, elevando os custos para o Sistema Único de Saúde (SUS) (Zhou, Qian et al. 2014, Machado, Cavalcanti et al. 2017, Zonta, Velasquez et al. 2018). Um estudo sobre a incidência, prevalência e mortalidade por sepse nas UTIs brasileiras mostrou que cerca de 200 mil pacientes adultos morrem por ano e que a taxa de mortalidade é tanto maior quanto menores são os recursos disponíveis no hospital (Machado, Cavalcanti et al. 2017).

Alguns dos problemas apontados por Machado e colegas (2017) são a falta de conhecimento da equipe de saúde, o que leva a diagnóstico e tratamento tardios, baixa disponibilidade de leitos nas unidades de terapia intensiva e infecções relacionadas à própria assistência, uma vez que esses pacientes necessitam de cuidados e, consequentemente, manipulações constantes, as quais podem levar a infecções se esses procedimentos não são adequadamente realizados.

O sítio respiratório ainda é uma das principais fontes de processos infecciosos. Pacientes sob ventilação mecânica e com internação prolongada fazem parte da população de pacientes graves dentro das UTIs (Júnior, David et al. 2006, De Freitas Caires, Gaudet et al. 2018). Indivíduos que tiveram sepse têm risco aumentado de ter uma nova infecção e uma expectativa de vida menor quando comparados com indivíduos que nunca tiveram sepse (Zhou, Qian et al. 2014, Machado, Cavalcanti et al. 2017).

A resposta imune do hospedeiro é um fator preponderante para infecções. Assim, algumas condições, como o envelhecimento, procedimentos invasivos, infecções

por micro-organismos multirresistentes aos antibióticos, desnutrição, alcoolismo e *diabetes mellitus*, podem aumentar as chances de infecção (Júnior, David et al. 2006).

Um estudo feito em um hospital público do Paraná mostrou que a prevalência de sepse foi maior no sexo masculino e em pacientes com idade superior a 70 anos. Outro dado importante é a origem da infecção; 50,2% das infecções nesta população têm origem nosocomial. Doenças crônicas como hipertensão arterial e doenças cardiovasculares foram evidentes nestes indivíduos (Zonta, Velasquez et al. 2018). Estas comorbidades também estão relacionadas com o desenvolvimento da sepse e suas complicações (Santos, Souza et al. 2016).

Fisiopatologia da sepse

Como a sepse é uma resposta multifacetada do hospedeiro a um patógeno infectante, o entendimento de sua patofisiologia é de fundamental importância no desenvolvimento de novas estratégias terapêuticas. Ao nível fisiológico, a sepse se desenvolve por meio de uma complexa cascata de eventos desencadeados por uma série de fatores que envolvem múltiplos mediadores. A invasão por patógenos inicia o recrutamento para a área afetada de vários tipos celulares, como macrófagos e células endoteliais e polimorfonucleares; a ativação destas células desencadeia a liberação de mediadores pró-inflamatórios que alteram a permeabilidade vascular e atraem mais células para a região. Os sistemas de coagulação e do complemento são ativados, produzindo mais citocinas quimioatrativas para as células polimorfonucleares e monócitos. A resposta inflamatória localizada consiste em vasodilatação, marginalização celular e alterações nas funções das células endoteliais, resultando em alteração na distribuição do fluxo sanguíneo, aumento na permeabilidade vascular e edema. Todo este processo tem o propósito de restaurar a função normal no tecido; no entanto, na sepse, esse mecanismo pode-se tornar excessivo, resultando em dano ao órgão, disfunção e morte (Vincent 2000).

Reologia do eritrócito na sepse

Na sepse, um aspecto fisiológico significante são as alterações hemodinâmicas e distúrbios na microcirculação, sendo estas mudanças relacionadas, em parte, aos efeitos cardíacos e vasculares de vários fatores gerados durante o curso da infecção (Ferguson and Brown 1996, Hinshaw 1996). Associado a isto, alguns estudos relatam a diminuição da deformabilidade de eritrócitos em casos de sepse (Machiedo, Powell et al. 1989,

Powell, Machiedo et al. 1991, Powell, Machiedo et al. 1993, Astiz, DeGent et al. 1995), o que também pode contribuir para as alterações hemodinâmicas. Danos microvasculares também ocorrem e levam a prejuízos na oxigenação de tecidos, favorecendo o pior desfecho em casos de sepse, que é a falência múltipla de órgãos (Hinshaw 1996).

Modificações no conteúdo ou na forma dos eritrócitos de pacientes sépticos vêm sendo descritos a alguns anos. Ácido siálico e glicoforina A foram quantificados em pacientes com sepse; os resultados obtidos mostraram que o conteúdo de ácido siálico foi menor nestes pacientes, mas sem alteração nos níveis de glicoforina A. Outro resultado relevante deste estudo foi que os eritrócitos dos pacientes sépticos eram mais esféricos (Piagnerelli, Boudjeltia et al. 2003).

Ao acompanhar pacientes com sepse internados em UTI desde o dia da admissão, já no primeiro e terceiro dia de internação foi possível observar diminuição na deformabilidade de eritrócitos, a qual foi mais grave nos pacientes não sobreviventes e, por isso, este resultado foi associado pelos autores à mortalidade. A agregação dessas células foi maior em pacientes com sepse, o que poderia afetar a microcirculação, juntamente com outras alterações presentes nos eritrócitos (Donadello, Piagnerelli et al. 2015).

Outro estudo realizado por Oliveira e colegas (2017), com 18 voluntários com sepse, verificou alterações na morfologia de eritrócitos, com predomínio de equinócitos e esferócitos, modificações estas que não foram observadas em voluntários saudáveis do grupo controle. Estas alterações foram associadas à produção de espécies reativas de oxigênio (ROS), que estão aumentadas no quadro séptico, levando a modificações na membrana de eritrócitos que poderiam acionar as células de defesa, mantendo o estado pró-oxidativo (Oliveira, Pontes-de-Carvalho et al. 2017).

Alguns estudos indicam que a reologia dos eritrócitos pode ser influenciada por muitos fatores, incluindo alterações nas concentrações intracelulares de cálcio, adenosina trifosfato (ATP) e 2,3-difosfoglicerato, efeitos do óxido nítrico e decréscimo de alguns componentes de membrana como ácido siálico. Outros fatores incluem interações com os leucócitos e seus produtos (ROS) ou ainda por efeito de variações de temperatura (Piagnerelli, Boudjeltia et al. 2003). No entanto, há ainda muito a se descobrir com relação a alterações reológicas dos eritrócitos na sepse e seus efeitos no fluxo sanguíneo e transporte de oxigênio. Neste sentido, novos achados podem ser

importantes no desenvolvimento de novas estratégias terapêuticas para melhorar a disponibilidade de oxigênio celular e, portanto, reduzir o risco de falência de órgãos.

Desordens hipertensivas da gestação

As desordens hipertensivas da gestação ocorrem quando os níveis pressóricos são iguais ou superiores a 140/90 mmHg, existindo em quatro formas: pré-eclâmpsia/eclâmpsia, hipertensão crônica, pré-eclâmpsia sobreposta à hipertensão crônica e hipertensão gestacional (Sibai 2002, Vest and Cho 2014, Guedes-Martins 2017).

A pré-eclâmpsia (PE) aparece após 20 semanas de gestação e é associada à proteinúria (300 mg de proteína por coleta de urina de 24 horas) ou na ausência de proteinúria é associada a algumas alterações como contagem de plaquetas, concentração de creatinina, doença renal, mudanças nos níveis de transaminases, edema pulmonar ou sintomas cerebrais e visuais. Ocorre em aproximadamente 2-5% da população grávida e apresenta alguns fatores de risco como idade superior a 40 anos, hiperlipidemia, histórico de gestação anterior acompanhada de hipertensão ou diabetes, obesidade, entre outros (Vest and Cho 2014).

A hipertensão crônica é diagnosticada antes da gestação ou antes de 20 semanas de gestação. Ocorre em 20% das mulheres grávidas e está associada ao aumento dos ricos para complicações pós-parto (Sibai 2002, Vest and Cho 2014). Já a pré-eclâmpsia sobreposta à hipertensão crônica é uma condição em que a paciente já era hipertensa podendo ou não desenvolver proteinúria após 20 semanas de gestação. Ocorre em cerca de 20-25% da população (Guedes-Martins 2017).

Muti e colegas (Muti, Tshimanga et al. 2015) mostraram que mulheres mais velhas teriam mais chances de desenvolver hipertensão durante a gravidez e este quadro hipertensivo aumentaria a chance de ter um bebê com baixo peso em comparação com grávidas sem hipertensão.

A hipertensão diagnosticada na gestação aumenta as chances de algumas complicações, como deslocamento de placenta, falência de órgãos, oligúria, lesão hepatocelular, edema pulmonar e trombocitopenia (Brichant, Dewandre et al. 2010, Matsuda, Hayashi et al. 2011, Kintiraki, Papakatsika et al. 2015).

Alguns fatores de risco vêm sendo associados a aumento da chance de desenvolvimento de alguma desordem hipertensiva durante a gestação. A idade materna

e o índice de massa corporal (Bener and Saleh 2013), o ganho de peso durante a gestação (Schneider, Freerksen et al. 2011) e o histórico familiar podem ser fatores preponderantes (Williams and Broughton Pipkin 2011).

Fisiopatologia das desordens hipertensivas da gravidez

A fisiopatologia das desordens hipertensivas ainda precisa ser elucida. A hipertensão gestacional, a pré-eclâmpsia sobreposta à hipertensão crônica e a pré-eclâmpsia são condições em que não é possível distinguir sua progressão porque seus processos podem se sobrepor, de tal forma que um pode ser o estágio inicial da outra (Naderi, Tsai et al. 2017, Ying, Catov et al. 2018). De fato, Barton e colegas mostraram que 46% das pacientes que desenvolveram hipertensão gestacional tiveram pré-eclâmpsia e desse percentual cerca de 10% progrediram para um quadro mais severo (Barton, O'Brien J et al. 2001).

A hipertensão crônica está associada com a restrição de crescimento do feto e ruptura placentária, além de aumentar as chances do desenvolvimento de pré-eclâmpsia. O que se propõe é que há um conjunto de variáveis maternas, fetais e placentárias que, juntas, contribuem para as alterações observadas nas diferentes desordens hipertensivas (Chandiramani and Shennan 2008, Naderi, Tsai et al. 2017, Ying, Catov et al. 2018).

Alterações em eritrócitos nas desordens hipertensivas da gravidez

Como visto no tópico anterior, as desordens hipertensivas da gestação são de definição complexa. Elas englobam um espectro de condições que muitas vezes são difíceis de diferenciar devido à existência de variações entre as próprias diretrizes internacionais (Rouse, Eckert et al. 2016). A despeito disso, essas condições clínicas envolvem alterações hemato-bioquímicas e/ou alterações hemorreológicas e hemodinâminas (Heilmann, Rath et al. 2004, Fodor, Gyorffy et al. 2011, Han, Liu et al. 2014, Chandi, Sirohiwal et al. 2015), como qualquer desordem hipertensiva. Logo, os eritrócitos, por serem as células mais abundantes do sangue, merecem atenção. Na pré-eclâmpsia, por exemplo, alterações relacionadas à inadequação na anatomia e fisiologia vascular materno-fetal, bem como a presença característica de hiperlipidemia e leucocitose neutrófila − que contribuem para o aumento na viscosidade sanguínea e outras propriedades hemodinâmicas − são fatores que podem favorecer a ocorrência de dano e senescência prematura de eritrócitos (Catarino, Rebelo et al. 2009).

De fato, Catarino e colegas encontraram aumento em marcadores de danos ao eritrócito em grávidas com PE, bem como maiores valores de RBC, Hb e Ht associados ao aumento da contagem de reticulócitos e do índice de produção de reticulócitos, sugerindo um maior estímulo eritropoiético – diante do aumento do dano/senescência e consequente remoção de eritrócitos – e indicando maior heterogeneidade de células na PE, em consequência da existência de grande quantidade de eritrócitos jovens entre aqueles que sofrem um processo acelerado de dano e envelhecimento e, possivelmente, redução na deformabilidade e funcionalidade (Catarino, Rebelo et al. 2009).

Heilmann e colegas relataram menor deformabilidade de eritrócitos em mulheres com PE grave, e chamaram atenção para o importante papel de parâmetros hemorreológicos na doença, especialmente considerando regiões de microcirculação com aumento da tensão de cisalhamento, como é o caso do espaço interviloso da placenta (Heilmann, Rath et al. 2004). Em outro estudo, tanto na PE como em alguns casos de restrição de crescimento intrauterino, uma menor deformabilidade de eritrócitos foi encontrada e foi ainda mais pronunciada em casos graves de complicações maternas e fetais, em que cerca de 5 dias após o parto ela assume valores característicos aos de mulheres não grávidas (Schauf, Lang et al. 2002).

Sabe-se também que os diferentes estados hipertensivos na gestação são associados a alterações morfológicas de eritrócitos, principalmente em pacientes com PE grave e hipertensão gestacional (Hernandez Hernandez, Villasenor et al. 2015).

Uma das possíveis razões para as alterações observadas nos eritrócitos de grávidas com desordens hipertensivas é a dislipidemia associada a essas condições. O conteúdo de colesterol e fosfolipídios da membrana é essencial para promover a fluidez, deformabilidade, estabilidade e funcionalidade dessas células (Chabanel, Flamm et al. 1983) e alterações nos lipídios circulantes podem influenciar na composição de membrana dos eritrócitos (Cooper 1974, Cooper, Arner et al. 1975, Ejima, Ijichi et al. 2000). Neste sentido, mulheres que desenvolvem pré-eclâmpsia apresentam níveis elevados de colesterol total, LDL-C e triglicérides em todos os trimestres da gravidez e também menores níveis de HDL-C durante o terceiro trimestre quando comparadas com normotensivas (Spracklen, Smith et al. 2014). Da mesma forma, a hipertensão gestacional também é associada à dislipidemia (Sahu, Abraham et al. 2009).

Um parâmetro que também pode estar alterado nas desordens hipertensivas da gestação e que tem relação com a composição lipídica de membrana é a estabilidade osmótica de eritrócitos (Aires Rodrigues de Freitas, Vieira da Costa et al. 2018).

De Freitas e colegas mostraram que mulheres com PE de ocorrência precoce apresentam aumento significante da estabilidade osmótica de eritrócitos em relação a gravidas normotensivas e à PE de ocorrência tardia – que é mais branda em relação à PE de ocorrência precoce. Neste estudo, os autores observaram uma correlação positiva entre o aumento da pressão arterial e a estabilidade de eritrócitos *in vitro*, sugerindo que uma seleção mecânica estaria ocorrendo. Assim, eritrócitos danificados devido ao estresse oxidativo característico da doença seriam mais constantemente removidos pelo estresse mecânico pressórico, restando uma população maior de reticulócitos e eritrócitos mais estáveis na circulação (Aires Rodrigues de Freitas, Vieira da Costa et al. 2018).

O teste de estabilidade osmótica de eritrócitos oferece uma série de parâmetros que se relacionam com parâmetros hematimétricos e bioquímicos (Penha-Silva, Firmino et al. 2007, de Freitas, de Oliveira et al. 2010, de Arvelos, Rocha et al. 2013, de Freitas, Marquez-Bernardes et al. 2013, Aires Rodrigues de Freitas, Vieira da Costa et al. 2018), em uma ampla rede de inter-relações cuja compreensão ainda carece de muito estudo, principalmente em doenças que afetam o sistema circulatório como um todo, como é o caso das doenças hipertensivas da gestação. Até o momento, não são conhecidos estudos que compararam a estabilidade osmótica de eritrócitos em diferentes desordens hipertensivas da gestação.

Considerações finais

A manutenção da constituição normal e a integridade da membrana plasmática são elementos essenciais para que os eritrócitos sejam capazes de exercer sua função no organismo. Na sepse, a deformabilidade e estabilidade destas células são alteradas, o que prejudica, principalmente, a oxigenação dos tecidos, levando à falência múltipla de órgãos. Nas desordens hipertensivas da gestação há uma gama de alterações já evidenciadas em relação ao eritrócito e sabe-se que estas alterações podem estar envolvidas na piora do quadro clínico da mãe e do bebê, em decorrência de prejuízos à microcirculação. Neste contexto, a estabilidade de membrana de eritrócitos ainda é pouco estudada e pode auxiliar na compreensão da fisiopatologia da doença e das alterações sofridas pelos eritrócitos. Este trabalho teve por objetivo proporcionar avanços no conhecimento da estabilidade de membrana de eritrócitos na sepse e nas desordens hipertensivas da gestação. Para isso, foi feita a avaliação da estabilidade osmótica do eritrócito, estabelecendo-se correlações com variáveis hematimétricas e

bioquímicas sanguíneas, com o intuito de compreender os fatores que afetam a estabilidade de eritrócitos nesta condição. Os resultados aqui encontrados podem contribuir para o desenvolvimento de novas estratégias terapêuticas, para o estabelecimento de novas condutas pelos profissionais de saúde e para a redução de morbimortalidade em pacientes com sepse.

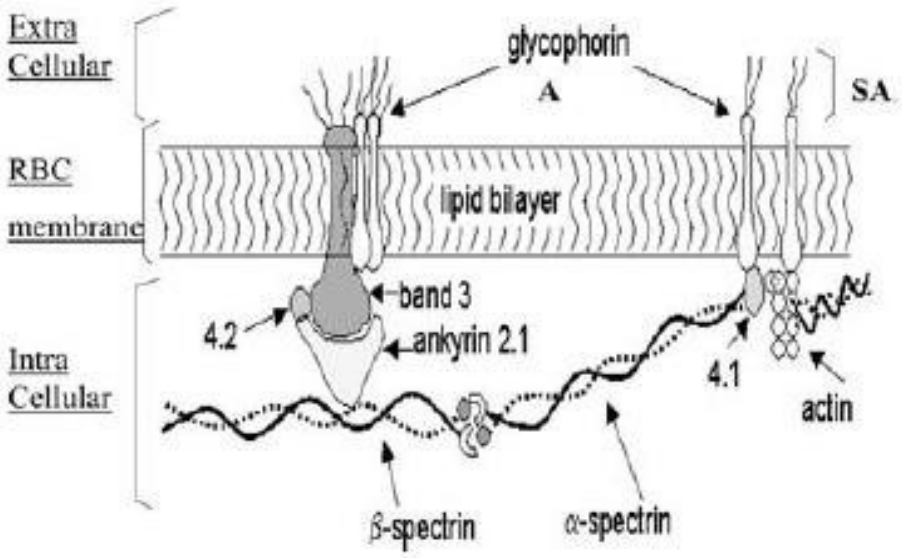

Figura 1. Representação esquemática das proteínas de membrana e do citoesqueleto do eritrócito humano. Fonte: PIAGNERELLI et al. (2003).

Referências

(2013). "Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy." Obstet Gynecol **122**(5): 1122-1131.

Abbaspour, N., R. Hurrell and R. Kelishadi (2014). "Review on iron and its importance for human health." J Res Med Sci. **19**(2): 164–174.

Aires Rodrigues de Freitas, M., A. Vieira da Costa, L. Alves de Medeiros, M. da Silva Garrote Filho, A. Lemos Debs Diniz and N. Penha-Silva (2018). "Are There Differences in the Anthropometric, Hemodynamic, Hematologic, and Biochemical Profiles between Late- and Early-Onset Preeclampsia?" Obstet Gynecol Int **2018**: 9628726. https://doi.org/10.1155/2018/9628726

Alves de Rezende, C. H., L. M. Coelho, L. M. Oliveira and N. Penha Silva (2009). "Dependence of the geriatric depression scores on age, nutritional status, and haematologic variables in elderly institutionalized patients." J Nutr Health Aging **13**(7): 617-621. https://doi.org/10.1007/s12603-009-0172-0

Angus, D. C. and T. van der Poll (2013). "Severe sepsis and septic shock." N Engl J Med **369**(9): 840-851. https://doi.org/10.1056/NEJMra1208623

Astiz, M. E., G. E. DeGent, R. Y. Lin and E. C. Rackow (1995). "Microvascular function and rheologic changes in hyperdynamic sepsis." Crit Care Med **23**(2): 265-271. https://doi.org/10.1097/00003246-199502000-00011

Bagdade, J. D., W. F. Buchanan, T. Pollare and H. Lithell (1995). "Abnormal lipoprotein phospholipid composition in patients with essential hypertension." Atherosclerosis **117**(2): 209-215. https://doi.org/10.1016/0021-9150(95)05573-F

Barton, J. R., M. O'Brien J, N. K. Bergauer, D. L. Jacques and B. M. Sibai (2001). "Mild gestational hypertension remote from term: progression and outcome." Am J Obstet Gynecol **184**(5): 979-983. https://doi.org/10.1067/mob.2001.112905

Barton, J. R. and B. M. Sibai (2004). "Diagnosis and management of hemolysis, elevated liver enzymes, and low platelets syndrome." Clin Perinatol **31**(4): 807-833, vii. https://doi.org/10.1016/j.clp.2004.06.008

Bateman, R., M. Sharpe, M. Singer and C. Ellis (2017). "The Effect of Sepsis on the Erythrocyte." International Journal of Molecular Sciences **18**(9): 1932. https://doi.org/10.3390/ijms18091932

Bateman, R. M. and K. R. Walley (2005). "Microvascular resuscitation as a therapeutic goal in severe sepsis." Critical Care **9**(Suppl 4): S27. https://doi.org/10.1186/cc3756

Bener, A. and N. M. Saleh (2013). "The impact of socio-economic, lifestyle habits, and obesity in developing of pregnancy-induced hypertension in fast-growing country: global comparisons." Clin Exp Obstet Gynecol **40**(1): 52-57.

Berhan, Y. (2016). "No Hypertensive Disorder of Pregnancy; No Preeclampsia-eclampsia; No Gestational Hypertension; No Hellp Syndrome. Vascular Disorder of Pregnancy Speaks for All." Ethiop J Health Sci **26**(2): 177-186. https://doi.org/10.4314/ejhs.v26i2.12

Bernardino Neto, M., E. B. de Avelar, Jr., T. S. Arantes, I. A. Jordao, J. C. da Costa Huss, T. M. de Souza, V. A. de Souza Penha, S. C. da Silva, P. C. de Souza, M. Tavares and N. Penha-Silva (2013). "Bivariate and multivariate analyses of the correlations between stability of the erythrocyte membrane, serum lipids and hematological variables." Biorheology **50**(5-6): 305-320. https://doi.org/10.3233/BIR-130641

Bone, R. C., R. A. Balk, F. B. Cerra, R. P. Dellinger, A. M. Fein, W. A. Knaus, R. M. Schein and W. J. Sibbald (1992). " Definitions for Sepsis and Organ Failure and Guidelines for the Use of Innovative Therapies in Sepsis." Chest **101**(6): 1644–1655. https://doi.org/10.1378/chest.101.6.1644

Bosmann, M. and P. A. Ward (2013). "The inflammatory response in sepsis." Trends Immunol **34**(3): 129-136. https://doi.org/10.1016/j.it.2012.09.004

Brichant, G., P. Y. Dewandre, J. M. Foidart and J. F. Brichant (2010). "Management of severe preeclampsia." Acta Clin Belg **65**(3): 163-169. https://doi.org/10.1179/acb.2010.035

Bujak, K., J. Wasilewski, T. Osadnik, S. Jonczyk, A. Kolodziejska, M. Gierlotka and M. Gasior (2015). "The Prognostic Role of Red Blood Cell Distribution Width in Coronary Artery Disease: A Review of the Pathophysiology." Dis Markers **2015**: 824624. https://doi.org/10.1155/2015/824624

Canessa, M., N. Adragna, H. S. Solomon, T. M. Connolly and D. C. Tosteson (1980). "Increased sodium-lithium countertransport in red cells of patients with essential hypertension." N Engl J Med **302**(14): 772-776. https://doi.org/10.1056/NEJM198004033021403

Carvalho, M. A., A. Faundes and L. C. Santos (1997). "Pregnancy-induced hypertension

and hyaline membrane disease." Int J Gynaecol Obstet **58**(2): 197-202. https://doi.org/10.1016/S0020-7292(97)00066-0

Catanzarite, V. A., S. M. Steinberg, C. A. Mosley, C. F. Landers, L. M. Cousins and J. M. Schneider (1995). "Severe preeclampsia with fulminant and extreme elevation of aspartate aminotransferase and lactate dehydrogenase levels: high risk for maternal death." Am J Perinatol **12**(5): 310-313. https://doi.org/10.1055/s-2007-994482

Catarino, C., I. Rebelo, L. Belo, P. Rocha-Pereira, S. Rocha, E. Bayer Castro, B. Patricio, A. Quintanilha and A. Santos-Silva (2009). "Erythrocyte changes in preeclampsia: relationship between maternal and cord blood erythrocyte damage." J Perinat Med **37**(1): 19-27. https://doi.org/10.1515/JPM.2009.007

Chabanel, A., M. Flamm, K. L. Sung, M. M. Lee, D. Schachter and S. Chien (1983). "Influence of cholesterol content on red cell membrane viscoelasticity and fluidity." Biophys J **44**(2): 171-176. https://doi.org/10.1016/S0006-3495(83)84288-X

Chandi, A., D. Sirohiwal and R. Malik (2015). "Association of early maternal hypertriglyceridemia with pregnancy-induced hypertension." Arch Gynecol Obstet **292**(5): 1135-1143. https://doi.org/10.1007/s00404-015-3706-1

Chandiramani, M. and A. Shennan (2008). "Hypertensive disorders of pregnancy: a UK-based perspective." Curr Opin Obstet Gynecol **20**(2): 96-101. https://doi.org/10.1097/GCO.0b013e3282f7336d

Chasis, J. A., P. Agre and N. Mohandas (1988). "Decreased membrane mechanical stability and in vivo loss of surface area reflect spectrin deficiencies in hereditary spherocytosis." J Clin Invest **82**(2): 617-623. https://doi.org/10.1172/JCI113640

Cherayil, B. J. (2015). "Pathophysiology of Iron Homeostasis during Inflammatory States." J Pediatr **167**(4 Suppl): S15-19. https://doi.org/10.1016/j.jpeds.2015.07.015

Churpek, M. M., F. J. Zadravecz, C. Winslow, M. D. Howell and D. P. Edelson (2015). "Incidence and Prognostic Value of the Systemic Inflammatory Response Syndrome and Organ Dysfunctions in Ward Patients." Am J Respir Crit Care Med **192**(8): 958-964. https://doi.org/10.1164/rccm.201502-0275OC

Cohen, R. M., R. S. Franco, P. K. Khera, E. P. Smith, C. J. Lindsell, P. J. Ciraolo, M. B. Palascak and C. H. Joiner (2008). "Red cell life span heterogeneity in

hematologically normal people is sufficient to alter HbA1c." Blood **112**(10): 4284-4291. https://doi.org/10.1182/blood-2008-04-154112

Cooper, R. (1974). "Modification of red cell membrane structure by cholesterol-Rich lipid dispersion." Journal of Clinical Investigation **55**: 115-196. https://doi.org/10.1172/JCI107901

Cooper, R. A. (1969). "Anemia with Spur Cells - a Red Cell Defect Acquired in Serum and Modified in Circulation." Journal of Clinical Investigation **48**(10): 1820-&. https://doi.org/10.1172/JCI106148

Cooper, R. A., E. C. Arner, J. S. Wiley and S. J. Shattil (1975). "Modification of red cell membrane structure by cholesterol-rich lipid dispersions. A model for the primary spur cell defect." J Clin Invest **55**(1): 115-126. https://doi.org/10.1172/JCI107901

Cooper, R. A., M. H. Leslie, S. Fischkoff, M. Shinitzky and S. J. Shattil (1978). "Factors influencing the lipid composition and fluidity of red cell membranes in vitro: production of red cells possessing more than two cholesterols per phospholipid." Biochemistry **17**(2): 327-331. https://doi.org/10.1021/bi00595a021

Cunha, C. C., L. R. Arvelos, J. O. Costa and N. Penha-Silva (2007). "Effects of glycerol on the thermal dependence of the stability of human erythrocytes." J Bioenerg Biomembr **39**(4): 341-347. https://doi.org/10.1007/s10863-007-9092-z

Custodio Afonso Rocha, V., L. Ramos de Arvelos, G. Pereira Felix, D. Nogueira Prado de Souza, M. Bernardino Neto, E. Santos Resende and N. Penha-Silva (2012). "Evolution of nutritional, hematologic and biochemical changes in obese women during 8 weeks after Roux-en-Y gastric bypasss." Nutr Hosp **27**(4): 1134-1140.

de Arvelos, L. R., V. C. Rocha, G. P. Felix, C. C. da Cunha, M. Bernardino Neto, M. da Silva Garrote Filho, C. de Fatima Pinheiro, E. S. Resende and N. Penha-Silva (2013). "Bivariate and multivariate analyses of the influence of blood variables of patients submitted to Roux-en-Y gastric bypass on the stability of erythrocyte membrane against the chaotropic action of ethanol." J Membr Biol **246**(3): 231-242. https://doi.org/10.1007/s00232-013-9524-0

De Freitas Caires, N., A. Gaudet, L. Portier, A. Tsicopoulos, D. Mathieu and P. Lassalle (2018). "Endocan, sepsis, pneumonia, and acute respiratory distress syndrome." Crit Care **22**(1): 280. https://doi.org/10.1186/s13054-018-2222-7

de Freitas, M. V., M. R. de Oliveira, D. F. dos Santos, R. de Cassia Mascarenhas Netto, S. B. Fenelon and N. Penha-Silva (2010). "Influence of the use of statin on the

stability of erythrocyte membranes in multiple sclerosis." J Membr Biol **233**(1-3): 127-134. https://doi.org/10.1007/s00232-010-9232-y

de Freitas, M. V., L. F. Marquez-Bernardes, L. R. de Arvelos, L. F. Paraiso, E. Goncalves, O. A. F., C. Mascarenhas Netto Rde, M. B. Neto, M. S. Garrote-Filho, P. C. de Souza and N. Penha-Silva (2014). "Influence of age on the correlations of hematological and biochemical variables with the stability of erythrocyte membrane in relation to sodium dodecyl sulfate." Hematology **19**(7): 424-430. https://doi.org/10.1179/1607845413Y.0000000145

de Freitas, M. V., L. F. Marquez-Bernardes, L. R. de Arvelos, L. F. Paraiso, E. O. A. F. Goncalves, R. D. Mascarenhas Netto, M. B. Neto, M. S. Garrote-Filho, P. C. de Souza and N. Penha-Silva (2013). "Influence of age on the correlations of hematological and biochemical variables with the stability of erythrocyte membrane in relation to sodium dodecyl sulfate." Hematology. https://doi.org/10.1179/1607845413Y.0000000145

de Freitas, M. V., L. F. Marquez-Bernardes, L. R. de Arvelos, L. F. Paraiso, E. O. A. F. Goncalves, C. Mascarenhas Netto Rde, M. B. Neto, M. S. Garrote-Filho, P. C. de Souza and N. Penha-Silva (2014). "Influence of age on the correlations of hematological and biochemical variables with the stability of erythrocyte membrane in relation to sodium dodecyl sulfate." Hematology **19**(7): 424-430. https://doi.org/10.1179/1607845413Y.0000000145

Deutschman, C. S. and K. J. Tracey (2014). "Sepsis: current dogma and new perspectives." Immunity **40**(4): 463-475. https://doi.org/10.1016/j.immuni.2014.04.001

Donadello, K., M. Piagnerelli, G. Reggiori, L. Gottin, S. Scolletta, G. Occhipinti, K. Zouaoui Boudjeltia and J. L. Vincent (2015). "Reduced red blood cell deformability over time is associated with a poor outcome in septic patients." Microvasc Res **101**: 8-14. https://doi.org/10.1016/j.mvr.2015.05.001

Ejima, J., T. Ijichi, Y. Ohnishi, T. Maruyama, Y. Kaji, S. Kanaya, T. Fujino, N. Uyesaka and T. Ohmura (2000). "Relationship of high-density lipoprotein cholesterol and red blood cell filterability: cross-sectional study of healthy subjects." Clin Hemorheol Microcirc **22**(1): 1-7.

Evans, E., N. Mohandas and A. Leung (1984). "Static and dynamic rigidities of normal and sickle erythrocytes." Journal of Clinical Investigation **73**: 477-488. https://doi.org/10.1172/JCI111234

Ferguson, K. L. and L. Brown (1996). "Bacteremia and sepsis." Emergency medicine clinics of North America **14**: 185–195. https://doi.org/10.1016/S0733-8627(05)70244-3

Fessler, M. B., K. Rose, Y. Zhang, R. Jaramillo and D. C. Zeldin (2013). "Relationship between serum cholesterol and indices of erythrocytes and platelets in the US population." J Lipid Res **54**(11): 3177-3188. https://doi.org/10.1194/jlr.P037614

Fleischmann, C., A. Scherag, N. K. J. Adhikari, C. S. Hartog, T. Tsaganos, P. Schlattmann, D. C. Angus and K. Reinhart (2016). "Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations." American Journal of Respiratory and Critical Care Medicine **193**(3): 259-272. https://doi.org/10.1164/rccm.201504-0781OC

Fodor, A., A. Gyorffy, L. Orosz and T. Major (2011). "[Hemorrheological changes and their clinical relevance in pre-eclampsia]." Orv Hetil **152**(6): 205-211. https://doi.org/10.1556/OH.2011.29031

Fonseca, L. C., L. R. Arvelos, R. C. Netto, A. B. Lins, M. S. Garrote-Filho and N. Penha-Silva (2010). "Influence of the albumin concentration and temperature on the lysis of human erythrocytes by sodium dodecyl sulfate." J Bioenerg Biomembr **42**(5): 413-418. https://doi.org/10.1007/s10863-010-9310-y

Garrote-Filho, M. S., M. Bernardino-Neto and N. Penha-Silva (2017). "Influence of Erythrocyte Membrane Stability in Atherosclerosis." Curr Atheroscler Rep **19**(4): 1-7. https://doi.org/10.1007/s11883-017-0653-2

Guedes-Martins, L. (2017). "Superimposed Preeclampsia." Adv Exp Med Biol **956**: 409-417. https://doi.org/10.1007/5584_2016_82

Han, L., X. Liu, H. Li, J. Zou, Z. Yang, J. Han, W. Huang, L. Yu, Y. Zheng and L. Li (2014). "Blood coagulation parameters and platelet indices: changes in normal and preeclamptic pregnancies and predictive values for preeclampsia." PLoS One **9**(12): e114488. https://doi.org/10.1371/journal.pone.0114488

Heilmann, L., W. Rath and K. Pollow (2004). "Hemorheological changes in women with severe preeclampsia." Clin Hemorheol Microcirc **31**(1): 49-58.

Hernandez Hernandez, J. D., O. R. Villasenor, J. Del Rio Alvarado, R. O. Lucach, A. Zarate, R. Saucedo and M. Hernandez-Valencia (2015). "Morphological changes of red blood cells in peripheral blood smear of patients with pregnancy-related hypertensive disorders." Arch Med Res **46**(6): 479-483. https://doi.org/10.1016/j.arcmed.2015.07.003

Hinshaw, L. B. (1996). "Sepsis/septic shock: participation of the microcirculation: an abbreviated review." Crit Care Med **24**(6): 1072-1078. https://doi.org/10.1097/00003246-199606000-00031

Hladky, S. B. and T. J. Rink (1978). "Osmotic behaviour of human red blood cells: an interpretation in terms of negative intracellular fluid pressure." J Physiol **274**: 437-446. https://doi.org/10.1113/jphysiol.1978.sp012158

Hofmeyr, G. J. and M. Belfort (2009). "Proteinuria as a predictor of complications of pre-eclampsia." BMC Med **7**: 11. https://doi.org/10.1186/1741-7015-7-11

Hotchkiss, R. S., G. Monneret and D. Payen (2013). "Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy." Nat Rev Immunol **13**(12): 862-874. https://doi.org/10.1038/nri3552

Janz, D. R., J. A. Bastarache, J. F. Peterson, G. Sills, N. Wickersham, A. K. May, L. J. Roberts and L. B. Ware (2013). "Association Between Cell-Free Hemoglobin, Acetaminophen, and Mortality in Patients With Sepsis." Critical Care Medicine **41**(3): 784-790. https://doi.org/10.1097/CCM.0b013e3182741a54

Jarolim, P., H. L. Rubin, D. Zakova, J. Storry and M. E. Reid (1998). "Characterization of seven low incidence blood group antigens carried by erythrocyte band 3 protein." Blood **92**(12): 4836-4843. https://doi.org/10.1182/blood.V92.12.4836

Jo, Y. H., K. Kim, J. H. Lee, C. Kang, T. Kim, H.-M. Park, K. W. Kang, J. Kim and J. E. Rhee (2013). "Red cell distribution width is a prognostic factor in severe sepsis and septic shock." The American Journal of Emergency Medicine **31**(3): 545-548. https://doi.org/10.1016/j.ajem.2012.10.017

Júnior, J. A. L. S., C. M. David, R. Hatum, P. C. S. P. Souza, A. Japiassú, C. T. S. Pinheiro, G. Friedman, O. B. da Silva, M. D. A. Dias, E. Koterba, F. S. Dias, C. Piras, G. d. E. d. S. d. F. AMIB and R. R. Ronir Raggio Luiz (2006). "Sepse Brasil: Estudo Epidemiológico da Sepse em Unidades de Terapia Intensiva Brasileiras." Revista Brasileira Terapia Intensiva **18**: 9-17. https://doi.org/10.1590/S0103-507X2006000100003

Kanakaraj, P. and M. Singh (1989). "Influence of hypercholesterolemia on morphological and rheological characteristics of erythrocytes." Atherosclerosis **76**(2-3): 209-218. https://doi.org/10.1016/0021-9150(89)90105-6

Kaukonen, K. M., M. Bailey, S. Suzuki, D. Pilcher and R. Bellomo (2014). "Mortality related to severe sepsis and septic shock among critically ill patients in Australia

and New Zealand, 2000-2012." JAMA **311**(13): 1308-1316. https://doi.org/10.1001/jama.2014.2637

Kintiraki, E., S. Papakatsika, G. Kotronis, D. G. Goulis and V. Kotsis (2015). "Pregnancy-Induced hypertension." Hormones (Athens) **14**(2): 211-223. https://doi.org/10.14310/horm.2002.1582

Kosalka, K., E. Wachowska and R. Slotwinski (2017). "Disorders of nutritional status in sepsis - facts and myths." Prz Gastroenterol **12**(2): 73-82. https://doi.org/10.5114/pg.2017.68165

Koter, M., I. Franiak, K. Strychalska, M. Broncel and J. Chojnowska-Jezierska (2004). "Damage to the structure of erythrocyte plasma membranes in patients with type-2 hypercholesterolemia." Int J Biochem Cell Biol **36**(2): 205-215. https://doi.org/10.1016/S1357-2725(03)00195-X

Lange, Y. and J. M. Slayton (1982). "Interaction of cholesterol and lysophosphatidylcholine in determining red cell shape." J Lipid Res **23**(8): 1121-1127. https://doi.org/10.1016/S0022-2275(20)38048-2

Leeman, L. and P. Fontaine (2008). "Hypertensive Disorders of Pregnancy." American Family Physician **78**: 93-100.

Lemos, G. S., L. F. Marquez-Bernardes, L. R. Arvelos, L. F. Paraiso and N. Penha-Silva (2011). "Influence of glucose concentration on the membrane stability of human erythrocytes." Cell Biochem Biophys **61**(3): 531-537. https://doi.org/10.1007/s12013-011-9235-z

Lewis, A. E. and J. Gershow (1961). "Estimation of erythrocyte life span from bilirubin clearance." J Appl Physiol **16**: 1140-1141. https://doi.org/10.1152/jappl.1961.16.6.1140

Liu, S. C. and J. Palek (1980). "Spectrin tetramer–dimer equilibrium and the stability of erythrocyte membrane skeletons." Nature **285**: 586–588. https://doi.org/10.1038/285586a0

Lurie, S. and Y. Mamet (2000). "Red blood cell survival and kinetics during pregnancy." Eur J Obstet Gynecol Reprod Biol **93**(2): 185-192. https://doi.org/10.1016/S0301-2115(00)00290-6

Machado, F. R., M. S. C. d. Assunção, A. B. Cavalcanti, A. M. Japiassú, L. C. P. d. Azevedo and M. C. Oliveira (2016). "Getting a consensus: advantages and disadvantages of Sepsis 3 in the context of middle-income settings." Revista

Brasileira de Terapia Intensiva **28**(4). https://doi.org/10.5935/0103-507X.20160068

Machado, F. R., A. B. Cavalcanti, F. A. Bozza, E. M. Ferreira, F. S. Angotti Carrara, J. L. Sousa, N. Caixeta, R. Salomao, D. C. Angus, L. C. Pontes Azevedo, S. Investigators and N. Latin American Sepsis Institute (2017). "The epidemiology of sepsis in Brazilian intensive care units (the Sepsis PREvalence Assessment Database, SPREAD): an observational study." Lancet Infect Dis **17**(11): 1180-1189. https://doi.org/10.1016/S1473-3099(17)30322-5

Machiedo, G. W., R. J. Powell, B. F. Rush, Jr., N. I. Swislocki and G. Dikdan (1989). "The incidence of decreased red blood cell deformability in sepsis and the association with oxygen free radical damage and multiple-system organ failure." Arch Surg **124**(12): 1386-1389. https://doi.org/10.1001/archsurg.1989.01410120032007

Magee, L. A., A. Pels, M. Helewa, E. Rey, P. von Dadelszen and S. H. G. Committee (2014). "Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy: executive summary." J Obstet Gynaecol Can **36**(7): 575-576. https://doi.org/10.1016/S1701-2163(15)30533-8

Malandrino, N., W. C. Wu, T. H. Taveira, H. B. Whitlatch and R. J. Smith (2012). "Association between red blood cell distribution width and macrovascular and microvascular complications in diabetes." Diabetologia **55**(1): 226-235. https://doi.org/10.1007/s00125-011-2331-1

Martin, S. L., S. Desai, R. Nanavati, R. B. Colah, K. Ghosh and M. B. Mukherjee (2018). "Red cell distribution width and its association with mortality in neonatal sepsis." J Matern Fetal Neonatal Med: 1-6. https://doi.org/10.1080/14767058.2017.1421932

Mascarenhas Netto, R. C., C. Fabbri, M. V. de Freitas, M. Bernardino Neto, M. S. Garrote-Filho, M. V. Lacerda, E. S. Lima and N. Penha-Silva (2014). "Influence of Plasmodium vivax malaria on the relations between the osmotic stability of human erythrocyte membrane and hematological and biochemical variables." Parasitol Res **113**(3): 863-874. https://doi.org/10.1007/s00436-013-3717-4

Matsuda, Y., K. Hayashi, A. Shiozaki, Y. Kawamichi, S. Satoh and S. Saito (2011). "Comparison of risk factors for placental abruption and placenta previa: case-cohort study." J Obstet Gynaecol Res **37**(6): 538-546. https://doi.org/10.1111/j.1447-0756.2010.01408.x

Mayr, F. B., S. Yende and D. C. Angus (2014). "Epidemiology of severe sepsis." Virulence **5**(1): 4-11. https://doi.org/10.4161/viru.27372

Metnitz, P. G., R. P. Moreno, E. Almeida, B. Jordan, P. Bauer, R. A. Campos, G. Iapichino, D. Edbrooke, M. Capuzzo, J. R. Le Gall and S. Investigators (2005). "SAPS 3--From evaluation of the patient to evaluation of the intensive care unit. Part 1: Objectives, methods and cohort description." Intensive Care Med **31**(10): 1336-1344. https://doi.org/10.1007/s00134-005-2762-6

Mittal, M., M. R. Siddiqui, K. Tran, S. P. Reddy and A. B. Malik (2014). "Reactive oxygen species in inflammation and tissue injury." Antioxid Redox Signal **20**(7): 1126-1167. https://doi.org/10.1089/ars.2012.5149

Mohandas, N., M. R. Clark, M. S. Jacobs and S. Shoehet (1980). "Analysis of Factors Regulating Erythrocytes Deformability." Journal of Clinical Investigation **66**: 563-573. https://doi.org/10.1172/JCI109888

Mohandas, N. and E. Evans (1994). "Mechanical Properties of the Red Cell Membrane in Relation to Molecular Structure and Genetic Defects." Annual Review of Biophysics and Biomolecular Structure **23**: 787-818. https://doi.org/10.1146/annurev.bb.23.060194.004035

Mohandas, N. and P. G. Gallagher (2008). "Red cell membrane: past, present, and future." Blood **112**(10): 3939-3948. https://doi.org/10.1182/blood-2008-07-161166

Mohanty, J. G., E. Nagababu and J. M. Rifkind (2014). "Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging." Front Physiol **5**: 84. https://doi.org/10.3389/fphys.2014.00084

Moralez, G. M., L. Rabello, T. C. Lisboa, M. Lima, R. M. Hatum, F. V. C. De Marco, A. Alves, J. Pinto, H. B. N. de Araujo, G. V. Ramos, A. R. Silva, G. C. Fernandes, G. B. A. Faria, C. L. Mendes, R. A. Ramos Filho, V. P. de Souza, P. do Brasil, F. A. Bozza, J. I. F. Salluh, M. Soares and O. S. Investigators (2017). "External validation of SAPS 3 and MPM0-III scores in 48,816 patients from 72 Brazilian ICUs." Ann Intensive Care **7**(1): 53. https://doi.org/10.1186/s13613-017-0276-3

Moreno, R. P., P. G. Metnitz, E. Almeida, B. Jordan, P. Bauer, R. A. Campos, G. Iapichino, D. Edbrooke, M. Capuzzo, J. R. Le Gall and S. Investigators (2005). "SAPS 3--From evaluation of the patient to evaluation of the intensive care unit. Part 2: Development of a prognostic model for hospital mortality at ICU admission." Intensive Care Med **31**(10): 1345-1355. https://doi.org/10.1007/s00134-005-2763-5

Moutzouri, A. G., A. T. Skoutelis, C. A. Gogos, Y. F. Missirlis and G. M. Athanassiou (2007). "Red blood cell deformability in patients with sepsis: a marker for prognosis and monitoring of severity." Clin Hemorheol Microcirc **36**(4): 291-299.

Muller, S., O. Ziegler, M. Donner, P. Drouin and J. F. Stoltz (1990). "Rheological properties and membrane fluidity of red blood cells and platelets in primary hyperlipoproteinemia." Atherosclerosis **83**(2-3): 231-237. https://doi.org/10.1016/0021-9150(90)90168-I

Murador, P. and E. Deffune (2007). "Aspectos estruturais da membrana eritrocitária." Revista Brasileira de Hematologia e Hemoterapia **29**: 168–178. https://doi.org/10.1590/S1516-84842007000200016

Mustafa, R., S. Ahmed, A. Gupta and R. C. Venuto (2012). "A comprehensive review of hypertension in pregnancy." J Pregnancy **2012**: 105918. https://doi.org/10.1155/2012/105918

Muti, M., M. Tshimanga, G. T. Notion, D. Bangure and P. Chonzi (2015). "Prevalence of pregnancy induced hypertension and pregnancy outcomes among women seeking maternity services in Harare, Zimbabwe." BMC Cardiovasc Disord **15**: 111. https://doi.org/10.1186/s12872-015-0110-5

Naderi, S., S. A. Tsai and A. Khandelwal (2017). "Hypertensive Disorders of Pregnancy." Curr Atheroscler Rep **19**(3): 15. https://doi.org/10.1007/s11883-017-0648-z

Naydenov, N. G. and A. I. Ivanov (2011). "Spectrin-adducin membrane skeleton: A missing link between epithelial junctions and the actin cytoskeletion?" Bioarchitecture **1**(4): 186-191. https://doi.org/10.4161/bioa.1.4.17642

Netto, R. D. M., C. Fabbri, M. V. de Freitas, M. B. Neto, M. S. Garrote, M. V. G. Lacerda, E. S. Lima and N. Penha-Silva (2014). "Influence of Plasmodium vivax malaria on the relations between the osmotic stability of human erythrocyte membrane and hematological and biochemical variables." Parasitology Research **113**(3): 863-874. https://doi.org/10.1007/s00436-013-3717-4

Oliveira, Y. P., L. C. Pontes-de-Carvalho, R. D. Couto and A. A. Noronha-Dutra (2017). "Oxidative stress in sepsis. Possible production of free radicals through an erythrocyte-mediated positive feedback mechanism." Braz J Infect Dis **21**(1): 19-26. https://doi.org/10.1016/j.bjid.2016.11.004

Orbach, A., O. Zelig, S. Yedgar and G. Barshtein (2017). "Biophysical and Biochemical Markers of Red Blood Cell Fragility." Transfus Med Hemother **44**(3): 183-187. https://doi.org/10.1159/000452106

Osterholm, E. A. and M. K. Georgieff (2015). "Chronic inflammation and iron metabolism." J Pediatr **166**(6): 1351-1357 e1351. https://doi.org/10.1016/j.jpeds.2015.01.017

Pacetti, D., R. Gagliardi, M. Balzano, N. G. Frega, M. L. Ojeda, M. Borrero, A. Ruiz and P. Lucci (2016). "Changes in the Fatty Acid Profile and Phospholipid Molecular Species Composition of Human Erythrocyte Membranes after Hybrid Palm and Extra Virgin Olive Oil Supplementation." J Agric Food Chem. https://doi.org/10.1021/acs.jafc.6b01903

Paraiso, L. F., M. V. de Freitas, E. O. A. F. Goncalves, O. P. de Almeida Neto, E. A. Pereira, R. C. Mascarenhas Netto, L. M. Cunha, M. Bernardino Neto, G. G. de Agostini, E. S. Resende and N. Penha-Silva (2014). "Influence of acute exercise on the osmotic stability of the human erythrocyte membrane." Int J Sports Med **35**(13): 1072-1077. https://doi.org/10.1055/s-0034-1371834

Paraiso, L. F., E. O. A. F. Goncalves, L. M. Cunha, O. P. de Almeida Neto, A. G. Pacheco, K. B. Araujo, M. D. Garrote-Filho, M. Bernardino Neto and N. Penha-Silva (2017). "Effects of acute and chronic exercise on the osmotic stability of erythrocyte membrane of competitive swimmers." PLoS One **12**(2): e0171318. https://doi.org/10.1371/journal.pone.0171318

Patel, H. H., H. R. Patel and J. M. Higgins (2015). "Modulation of red blood cell population dynamics is a fundamental homeostatic response to disease." Am J Hematol **90**(5): 422-428. https://doi.org/10.1002/ajh.23982

Patel, K. V., L. Ferrucci, W. B. Ershler, D. L. Longo and J. M. Guralnik (2009). "Red blood cell distribution width and the risk of death in middle-aged and older adults." Arch Intern Med **169**(5): 515-523. https://doi.org/10.1001/archinternmed.2009.11

Patel, K. V., J. G. Mohanty, B. Kanapuru, C. Hesdorffer, W. B. Ershler and J. M. Rifkind (2013). "Association of the red cell distribution width with red blood cell deformability." Adv Exp Med Biol **765**: 211-216. https://doi.org/10.1007/978-1-4614-4989-8_29

Patel, K. V., R. D. Semba, L. Ferrucci, A. B. Newman, L. P. Fried, R. B. Wallace, S. Bandinelli, C. S. Phillips, B. Yu, S. Connelly, M. G. Shlipak, P. H. Chaves, L. J. Launer, W. B. Ershler, T. B. Harris, D. L. Longo and J. M. Guralnik (2010). "Red cell distribution width and mortality in older adults: a meta-analysis." J Gerontol A Biol Sci Med Sci **65**(3): 258-265. https://doi.org/10.1093/gerona/glp163

Penha-Silva, N., L. R. Arvelos, C. C. Cunha, T. A. Aversi-Ferreira, L. F. Gouvea-e-Silva, M. S. Garrote-Filho, C. J. Finotti, M. Bernardino-Neto and F. G. de Freitas Reis (2008). "Effects of glycerol and sorbitol on the thermal dependence of the lysis of human erythrocytes by ethanol." Bioelectrochemistry **73**(1): 23-29. https://doi.org/10.1016/j.bioelechem.2008.04.002

Penha-Silva, N., C. B. Firmino, F. G. de Freitas Reis, J. C. da Costa Huss, T. M. de Souza, M. V. de Freitas and R. C. Netto (2007). "Influence of age on the stability of human erythrocyte membranes." Mech Ageing Dev **128**(7-8): 444-449. https://doi.org/10.1016/j.mad.2007.06.007

Pereira, K. R., R. F. Souza, S. L. Arantes and S. E. Moreno (2007). "Sepse: Epidemiologia, Fisiopatologia e Tratamento." Multitemas: 189-206.

Perlstein, T. S., J. Weuve, M. A. Pfeffer and J. A. Beckman (2009). "Red blood cell distribution width and mortality risk in a community-based prospective cohort." Arch Intern Med **169**(6): 588-594. https://doi.org/10.1001/archinternmed.2009.55

Piagnerelli, M., K. Z. Boudjeltia, M. Vanhaeverbeek and J. L. Vincent (2003). "Red blood cell rheology in sepsis." Intensive Care Med **29**(7): 1052-1061. https://doi.org/10.1007/s00134-003-1783-2

Powell, R. J., G. W. Machiedo and B. F. Rush, Jr. (1993). "Decreased red blood cell deformability and impaired oxygen utilization during human sepsis." Am Surg **59**(1): 65-68.

Powell, R. J., G. W. Machiedo, B. F. Rush, Jr. and G. Dikdan (1991). "Oxygen free radicals: effect on red cell deformability in sepsis." Crit Care Med **19**(5): 732-735. https://doi.org/10.1097/00003246-199105000-00022

Quintano Neira, R. A., S. Hamacher and A. M. Japiassu (2018). "Epidemiology of sepsis in Brazil: Incidence, lethality, costs, and other indicators for Brazilian Unified Health System hospitalizations from 2006 to 2015." PLoS One **13**(4): e0195873. https://doi.org/10.1371/journal.pone.0195873

Ramanadham, M. and S. S. Kaplay (1982). "Erythrocyte osmotic fragility in protein-energy malnutrition: cholesterol, phospholipid, and CA2+, Mg2+ adenosine triphosphatase." Biochem Med **27**(2): 226-231. https://doi.org/10.1016/0006-2944(82)90025-4

Rodrigues, R., L. A. de Medeiros, L. M. Cunha, M. D. S. Garrote-Filho, M. Bernardino Neto, P. T. Jorge, E. S. Resende and N. Penha-Silva (2018). "Correlations of the glycemic variability with oxidative stress and erythrocytes membrane stability in

patients with type 1 diabetes under intensive treatment." Diabetes Res Clin Pract **144**: 153-160. https://doi.org/10.1016/j.diabres.2018.01.031

Rouse, C. E., L. O. Eckert, B. J. Wylie, D. J. Lyell, A. Jeyabalan, S. Kochhar, T. F. McElrath and G. Brighton Collaboration Preeclampsia Working (2016). "Hypertensive disorders of pregnancy: Case definitions & guidelines for data collection, analysis, and presentation of immunization safety data." Vaccine **34**(49): 6069-6076. https://doi.org/10.1016/j.vaccine.2016.03.038

Sahu, S., R. Abraham, R. Vedavalli and M. Daniel (2009). "Study of lipid profile, lipid peroxidation and vitamin E in pregnancy induced hypertension." Indian J Physiol Pharmacol **53**(4): 365-369.

Santos, A. M., G. R. B. Souza and A. M. L. Oliveira (2016). "Sepse em adultos na unidade de terapia intensiva: características clínicas. ." Arquivos médicos dos Hospitais e da Faculdade de Ciências Médicas da Santa Casa de São Paulo **61**: 3-7.

Savitz, D., V. W. Sidel and A. K. Solomon (1964). "Osmotic Properties of Human Red Cells." J Gen Physiol. **48**(1): 79–94. https://doi.org/10.1085/jgp.48.1.79

Schauf, B., U. Lang, P. Stute, S. Schneider, K. Dietz, B. Aydeniz and D. Wallwiener (2002). "Reduced red blood cell deformability, an indicator for high fetal or maternal risk, is found in preeclampsia and IUGR." Hypertens Pregnancy **21**(2): 147-160. https://doi.org/10.1081/PRG-120005387

Schneider, S., N. Freerksen, H. Maul, S. Roehrig, B. Fischer and B. Hoeft (2011). "Risk groups and maternal-neonatal complications of preeclampsia--current results from the national German Perinatal Quality Registry." J Perinat Med **39**(3): 257-265. https://doi.org/10.1515/jpm.2011.010

Semba, R. D., K. V. Patel, L. Ferrucci, K. Sun, C. N. Roy, J. M. Guralnik and L. P. Fried (2010). "Serum antioxidants and inflammation predict red cell distribution width in older women: the Women's Health and Aging Study I." Clin Nutr **29**(5): 600-604. https://doi.org/10.1016/j.clnu.2010.03.001

Shamshirsaz, A. A., M. Paidas and G. Krikun (2012). "Preeclampsia, hypoxia, thrombosis, and inflammation." J Pregnancy **2012**: 374047. https://doi.org/10.1155/2012/374047

Shankar-Hari, M., D. A. Harrison, G. D. Rubenfeld and K. Rowan (2017). "Epidemiology of sepsis and septic shock in critical care units: comparison between sepsis-2 and sepsis-3 populations using a national critical care database." Br J Anaesth **119**(4): 626-636. https://doi.org/10.1093/bja/aex234

Sibai, B. M. (2002). "Chronic hypertension in pregnancy." Obstet Gynecol. **100**(2): 369-377. https://doi.org/10.1016/S0029-7844(02)02128-2

Singer, M., V. De Santis, D. Vitale and W. Jeffcoate (2004). "Multiorgan failure is an adaptive, endocrine-mediated, metabolic response to overwhelming systemic inflammation." The Lancet **364**(9433): 545-548. https://doi.org/10.1016/S0140-6736(04)16815-3

Singer, M., C. S. Deutschman, C. W. Seymour, M. Shankar-Hari, D. Annane, M. Bauer, R. Bellomo, G. R. Bernard, J.-D. Chiche, C. M. Coopersmith, R. S. Hotchkiss, M. M. Levy, J. C. Marshall, G. S. Martin, S. M. Opal, G. D. Rubenfeld, T. van der Poll, J.-L. Vincent and D. C. Angus (2016). "The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)." Jama **315**(8): 801. https://doi.org/10.1001/jama.2016.0287

Spracklen, C. N., C. J. Smith, A. F. Saftlas, J. G. Robinson and K. K. Ryckman (2014). "Maternal hyperlipidemia and the risk of preeclampsia: a meta-analysis." Am J Epidemiol **180**(4): 346-358. https://doi.org/10.1093/aje/kwu145

Straat, M., R. van Bruggen, D. de Korte and N. P. Juffermans (2012). "Red blood cell clearance in inflammation." Transfus Med Hemother **39**(5): 353-361. https://doi.org/10.1159/000342229

Sutera, S. P., R. A. Gardner, C. W. Boylan, G. L. Carroll, K. C. Chang, J. S. Marvel, C. Kilo, B. Gonen and J. R. Williamson (1985). "Age-related changes in deformability of human erythrocytes." Blood **65**(2): 275-282. https://doi.org/10.1182/blood.V65.2.275.275

Troeger, C., W. Holzgreve, A. Ladewig, X. Y. Zhong and S. Hahn (2006). "Examination of maternal plasma erythropoietin and activin A concentrations with regard to circulatory erythroblast levels in normal and preeclamptic pregnancies." Fetal Diagn Ther **21**(1): 156-160. https://doi.org/10.1159/000089068

Tsuda, K., Y. Minatogawa, S. Tsuda, H. Shima, I. Nishio, R. Kido and Y. Masuyama (1987). "Alterations in calcium-handling of erythrocytes in spontaneously hypertensive rats. Calcium-induced changes in the osmotic fragility of erythrocytes in hypertension." Jpn Heart J **28**(6): 923-931. https://doi.org/10.1536/ihj.28.923

Tziakas, D., G. Chalikias, A. Grapsa, T. Gioka, I. Tentes and S. Konstantinides (2012). "Red blood cell distribution width: a strong prognostic marker in cardiovascular disease: is associated with cholesterol content of erythrocyte membrane." Clin Hemorheol Microcirc **51**(4): 243-254. https://doi.org/10.3233/CH-2012-1530

Tziakas, D. N., G. K. Chalikias, D. Stakos and H. Boudoulas (2010). "The role of red blood cells in the progression and instability of atherosclerotic plaque." Int J Cardiol **142**(1): 2-7. https://doi.org/10.1016/j.ijcard.2009.10.031

Vats, K. and M. Paul (2016). "Study of fetal outcome in hypertensive disorders of pregnancy in a tertiary care maternity hospital of Delhi." Int J Reprod Contracept Obstet Gynecol. **5**(11): 3773-3777. https://doi.org/10.18203/2320-1770.ijrcog20163494

Vaya, A., A. Sarnago, O. Fuster, R. Alis and M. Romagnoli (2015). "Influence of inflammatory and lipidic parameters on red blood cell distribution width in a healthy population." Clin Hemorheol Microcirc **59**(4): 379-385. https://doi.org/10.3233/CH-141862

Vazquez-Rodriguez, J. G., C. D. Rios-Gutierrez, E. P. Paredes-Lozano and A. Garcia-Flores (2016). "[Frequency and maternal complications of the criteria of hemolysis in preeclamptic patients with HELLP syndrome treated in an intensive care unit]." Ginecol Obstet Mex **84**(1): 19-26.

Vest, A. R. and L. S. Cho (2014). "Hypertension in pregnancy." Curr Atheroscler Rep **16**(3): 395. https://doi.org/10.1007/s11883-013-0395-8

Vincent, J. L. (2000). "Update on sepsis: pathophysiology and treatment." Acta Clin Belg **55**(2): 79-87. https://doi.org/10.1080/17843286.2000.11754275

Williams, P. J. and F. Broughton Pipkin (2011). "The genetics of pre-eclampsia and other hypertensive disorders of pregnancy." Best Pract Res Clin Obstet Gynaecol **25**(4): 405-417. https://doi.org/10.1016/j.bpobgyn.2011.02.007

Ying, W., J. M. Catov and P. Ouyang (2018). "Hypertensive Disorders of Pregnancy and Future Maternal Cardiovascular Risk." J Am Heart Assoc **7**(17): e009382. https://doi.org/10.1161/JAHA.118.009382

Zalawadiya, S. K., V. Veeranna, S. S. Panaich and L. Afonso (2012). "Red cell distribution width and risk of peripheral artery disease: analysis of National Health and Nutrition Examination Survey 1999-2004." Vasc Med **17**(3): 155-163. https://doi.org/10.1177/1358863X12442443

Zalawadiya, S. K., H. Zmily, J. Farah, S. Daifallah, O. Ali and J. K. Ghali (2011). "Red cell distribution width and mortality in predominantly African-American population with decompensated heart failure." J Card Fail **17**(4): 292-298. https://doi.org/10.1016/j.cardfail.2010.11.006

Zhou, J., C. Qian, M. Zhao, X. Yu, Y. Kang, X. Ma, Y. Ai, Y. Xu, D. Liu, Y. An, D. Wu, R. Sun, S. Li, Z. Hu, X. Cao, F. Zhou, L. Jiang, J. Lin, E. Mao, T. Qin, Z. He, L. Zhou, B. Du and G. China Critical Care Clinical Trials (2014). "Epidemiology and outcome of severe sepsis and septic shock in intensive care units in mainland China." PLoS One **9**(9): e107181. https://doi.org/10.1371/journal.pone.0107181

Zicha, J., J. Kunes and M. A. Devynck (1999). "Abnormalities of membrane function and lipid metabolism in hypertension: a review." Am J Hypertens **12**(3): 315-331. https://doi.org/10.1016/S0895-7061(98)00178-2

Zonta, F. N. S., P. G. A. Velasquez, L. G. Velasquez, L. S. Demetrio, D. Miranda and M. C. B. D. Silva (2018). "Características epidemiológicas e clínicas da sepse em um hospital público do Paraná." Revista de Epidemiologia e Controle de Infecção **8**(3). https://doi.org/10.17058/reci.v8i3.11438

Zurauskaite, G., M. Meier, A. Voegeli, D. Koch, S. Haubitz, A. Kutz, L. Bernasconi, A. Huber, M. Bargetzi, B. Mueller and P. Schuetz (2018). "Biological pathways underlying the association of red cell distribution width and adverse clinical outcome: Results of a prospective cohort study." PLoS One **13**(1): e0191280. https://doi.org/10.1371/journal.pone.0191280

CAPÍTULO 2

Osmotic stability of human erythrocytes in sepsis and septic chock

Osmotic stability of human erythrocytes in sepsis and septic chock

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Abstract

Introduction. Sepsis is an inflammatory syndrome with severe organic dysfunction. This study aimed to evaluate the osmotic stability of erythrocytes in volunteers with sepsis or septic shock in relation to individuals without the disease.

Material and methods. The study included a total of 99 male volunteers, 49 healthy subjects, 25 with sepsis and 25 with septic shock. The osmotic stability of the erythrocyte membrane was characterized by the concentration of NaCl in which hyposmotic lysis begins (H_0) and reaches 50% (H_{50}) and 100% (H_{100}) of the erythrocyte population, and also by the variation in saline concentration between H_0 and H_{100} (dX).

Results. Lower values of H_0 , H_{50} and H_{100} , which means greater osmotic stability of erythrocytes; and lower levels of hemoglobin, erythrocyte counts and values of hematocrit, mean corpuscular hemoglobin and/or mean corpuscular hemoglobin concentration, indicating a tendency to anemia or anemia; and higher values of red-cell distribution width (RDW), were found in sepsis and septic shock. Positive associations were found between variables of erythrocyte stability, anemia and inflammation, and between RDW values and death risk.

Conclusions. The higher osmotic stability of erythrocytes in this population was associated with anemia and inflammation, and higher RDW values were associated with a greater chance of death.

Keywords: red blood cells, osmotic fragility test, inflammation, cell membrane, red cell distribution width, RDW.

Introduction

Sepsis is a syndrome characterized by exacerbation of metabolic and immune responses to an infection that affects the functioning of various organs. The septic profile has high morbidity and mortality in the Intensive Care Units, mainly in developing countries (Bone, Balk et al. 1992, Singer, Deutschman et al. 2016). Around 5.3 million people in the world die each year from sepsis; in Brazil this number would correspond to 233,409 adult patients (Fleischmann, Scherag et al. 2016, Machado, Assunção et al. 2016).

During the sepsis the body recruits immune mediators and defense cells, such as lymphocytes and monocytes, and stimulates the production of cytokines to fight inflammation. These changes alter vascular permeability, increase the levels of reactive oxygen species and modify the deformability capacity of erythrocytes (Donadello, Piagnerelli et al. 2015, Bateman, Sharpe et al. 2017).

In sepsis, erythrocytes are exposed to different microenvironments. Changes in the shape, decrease of 2,3-bisphosphoglycerate and increased affinity of hemoglobin for oxygen affect the function of erythrocytes. Another observed change is that patients with sepsis present an increase in hemolysis, with a consequent increase in free hemoglobin, and this has been associated with organic dysfunction and increased mortality (Bateman and Walley 2005, Janz, Bastarache et al. 2013, Bateman, Sharpe et al. 2017).

An important hematologic parameter associated with the prognosis of sepsis is the red cell distribution width (RDW). Several studies have shown that the increase in RDW could be a marker of mortality in patients with a variety of conditions, such as sepsis and septic shock (Jo, Kim et al. 2013, Martin, Desai et al. 2018).

The aim of this study was to evaluate the osmotic stability of erythrocytes in sepsis and septic shock, and its possible associations with hematimetric and biochemical blood variables, in order to better understand the effects of sepsis on red blood cells.

Material and Methods

Population

The study plan was approved by the Ethics Committee on Human Research of the Federal University of Uberlândia (nº 153.331/2012). Blood samples were obtained

after 48 hours after the diagnosis of sepsis or septic shock in tubes containing K_3EDTA (for hematologic analysis and determination of erythrocyte stability) and in tubes without anticoagulant (for biochemical analysis) (Vacutainer; Becton Dickinson, Juiz de Fora, MG, Brazil). These collections were carried out in patients admitted to the Clinical Hospital of the Federal University of Uberlândia, between 2015 and 2016. The study included 49 healthy volunteers (control group), 25 patients with sepsis (sepsis group) and 25 patients with septic shock (septic shock group).

Determination of the osmotic stability of human erythrocytes

The determination of the osmotic stability of erythrocytes was performed as described by Penha-Silva and colleagues (Penha-Silva, Firmino et al. 2007). Initially, duplicate sets of test tubes containing 1 mL of 0.1-1.5 g/dL NaCl solutions (Labsynth, Diadema, SP, Brazil) were preincubated at 37 °C for 10 min. After addition of 10 μL of whole blood and gentle shaking, the tubes were incubated at 37 °C for 30 min. After centrifugation at 1500 x *g* for 10 min (Hitachi Koki™, model CFR15XRII, Hitachinaka, Japan), the supernatants were used to evaluate the optical density at 540 nm (A_{540}) on a UV-VIS spectrophotometer (Shimadzu™, model UV1650TC, Japan). The graphs of A_{540} versus NaCl (X) concentration were adjusted by non-linear regression, according to the Boltzmann equation:

$$A_{540} = \frac{A_{max} - A_{min}}{1 + e^{(X - H_{50})/dX}} + A_{min} \quad (1)$$

where A_{max} and A_{min} represent respectively the maximum and minimum plateaus of A_{540}, H_{50} is the concentration of NaCl capable of promoting 50% hemolysis, and dX is equivalent to one-fourth of the variation in NaCl concentration responsible for 100% hemolysis.

The saline concentrations where the hyposmotic lysis begins (H_0) and end (H_{100}) were determined using the equations $H_0 = H_{50} + 4dX/2$ and $H_{100} = H_{50} - 4dX/2$, respectively.

Determination of hematologic and biochemical parameters

Hematologic parameters were obtained by means of an automated system (Sysmex K4500; Sysmex Corporation™, Mundelein, IL, USA). These parameters include red blood cell count (RBC), hematocrit (Ht), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular

hemoglobin concentration (MCHC), red-cell distribution width (RDW), and white blood cell (WBC) and platelet counts (Plt).

Biochemical parameters were measured using an automated analyzer (Architect c8000, IL, USA). These parameters include triglycerides (TGC), total cholesterol (t-C), low density lipoprotein cholesterol (VLDL-C), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), creatinine (Cr), sodium (Na^+), potassium (K^+), lactate, C-reactive protein (CRP) and arterial gasometry, including hydrogen potential (pH), partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (pCO_2), bicarbonate (HCO_3^-), base excess (BE) and oxygen saturation (SaO_2).

The reference values were: RBC, 4.3-5.7 x 10^6 /mm^3; Hb, 13.5-17.5 g/dL; Ht, 39-50%; MCV, 81-95 fL; MCH, 26-34 pg; MCHC, 31- 36 g/dL; RDW, 12-15 %; Plt, 150-450 x 10^3/mm^3; t-C, <200 (desirable) e ≥240 mg/dL (elevated); TGC, <150 (desirable) and >201 mg/dL (elevated); VLDL-C, <30 mg/dL (desirable); LDL-C, <100 (optimum) e >160 mg/dL (elevated); HDL-C, <45 (low) e >65 mg/dL (ideal); urea, 10-45 mg/dL; creatinine, 0.7-1.2 mg/dL; Na^+, 135-145 mEq/L; K^+, 3.7-5.6 mEq/L; PCR, <0,5mg/dL; lactate, 0.36-0.75 mmol/L; pH, 7.35-7.45; pO_2, 80 a 100 mmHg; pCO_2, 35-45 mmHg; HCO_3^-, 22-26 mEq/L; and SO_2, 94-98%.

Determination of risk of death

The risk of death was evaluated using the SAP3 (*Simplified Acute Physiology Score*) instrument (Metnitz, Moreno et al. 2005, Moreno, Metnitz et al. 2005) in its validated version for the Brazilian population (Moralez, Rabello et al. 2017).

Statistical analysis

The Shapiro-Wilk test was used to evaluate the normality of the data in relation to the type of diagnosis. Comparisons between the control, sepsis and septic shock groups were performed using One-Way ANOVA when the variables of the three groups showed normal distribution. For the variables in which one or all groups presented non- normal distribution, comparisons were made using the Kruskall-Wallis test. The comparisons made only between the sepsis and septic shock groups were made using the Student's t-test for two independent populations when the variables data of both groups presented normal distribution. For the variables that presented non-normal

distribution in one or both groups, comparisons were made using the Mann-Whitney test.

The existence of associations between the studied variables was analyzed using the Spearman correlation test, with correlations with p values < 0.05 being considered significant.

Results

Figure 1 shows a typical sigmoid constructed for determination of the osmotic stability parameters used in this study.

The baseline characteristics of the control, sepsis and septic shock groups are shown in **Table 1**. The absence of age difference between the groups indicates that there was good pairing. There was no statistically significant difference between the sepsis and septic shock groups for any of the variables considered, but there were many significant differences between the sepsis and/or septic shock groups in comparison to the control group for several of the studied variables.

In relation to erythrocyte stability, the sepsis and septic shock groups presented greater osmotic stability than the control group. The lower H_0 , H_{50} and H_{100} values found in both the sepsis group and the septic shock group in relation to the control group indicate unequivocally that individuals with these pathological conditions have erythrocytes that are osmotically more stable. This difference in behavior between erythrocytes of the two diseased populations in relation to the control group can be easily visualized in the graph shown in **Figure 2**, where the osmotic fragility curve of populations with sepsis and septic shock, constructed from the set of points of all individuals of these populations, are displaced to the left, i.e., towards lower saline concentrations, in relation to the curve obtained for the universe of individuals of the control group.

The lower Amin values found in both the sepsis group and the septic shock group compared to the control group (**Table 1**) indicate that erythrocytes from the two diseased populations were already more stable even under isosmotic conditions with blood.

The origin of this higher stability is certainly associated with the lower values of Amax observed in the sepsis and septic shock groups in relation to the control group (**Table 1**). This behavior can also be easily visualized in Figure 2. This difference in the groups is due to the existence of a tendency to anemia or anemia in the diseased

populations, which had lower red blood cell (RBC) counts, lower hemoglobin levels (Hb) and lower values of hematocrit (Ht), mean corpuscular hemoglobin (MCH) and/or mean corpuscular hemoglobin concentration (MCHC) in relation to the control group (**Table 1**). Lower concentrations of Hb mean less osmotic pressure and greater capacity of the erythrocyte to undergo volume expansion before a medium of hyposmotic nature (Savitz, Sidel et al. 1964, Hladky and Rink 1978), and this is what gives erythrocytes greater osmotic stability.

The greater stability of erythrocytes in both diseased groups can also be demonstrated by the significantly higher values of dX observed in both the sepsis group and the septic shock group in relation to the control group (**Table 1**).

The anemic picture of sick volunteers is certainly the result of malnutrition. The lowest values of total cholesterol (t-C), LDL-C (LDL-C) and HDL-C (HDL-C) observed in the sepsis and/or septic shock groups (**Table 1**) are certainly manifestations of this malnutrition state.

The highest values of red-cell distribution width (RDW) observed in both sepsis and septic shock (**Table 1**), are certainly associated with the anemic state of the volunteers, and should have some prognostic significance in these pathological conditions, since elevation in RDW has been shown to have a great prognostic capacity of the clinical outcome in several diseases. (Perlstein, Weuve et al. 2009, Patel, Semba et al. 2010, Bujak, Wasilewski et al. 2015, Vaya, Sarnago et al. 2015, Zurauskaite, Meier et al. 2018).

Algumas variáveis foram analisadas somente nos grupos sepse e choque séptico. Estas variáveis estão mostradas na **Tabela 2**. Também em relação a estas variáveis não houve diferença significante entre os grupos sepse e choque séptico. Como esses dois grupos não apresentaram diferenças também em suas características de base, eles foram agrupados em um único grupo de estudo para avaliação das associações existentes entre as variáveis estudadas por análise de correlação.

A better understanding of the origin of the changes shown in **Table 1** can be achieved by analyzing the correlations between the studied variables. The main correlations involving osmotic stability and hematological variables, especially RBC and RDW, in the three study groups are shown in **Table 3**.

Some of these correlations deserve attention. The negative correlation observed between dX and Amin in the sepsis and septic shock groups indicates that lower hemoglobin content would increase erythrocyte resistance against lysis as the saline

concentration of the medium decreases. The significant negative correlation observed between blood hemoglobin and C-reactive protein (CRP) levels in the septic shock group indicates that the inflammatory condition of the disease is associated with a decrease in blood hemoglobin levels. The strong correlation observed between mean corpuscular volume (MCV) and mean corpuscular hemoglobin (HCM) illustrates a known association. The inverse correlation of H_{50} with blood levels of t-C and LDL-C in the control group but not in the diseased population shows that the influence of lipidemia on the erythrocyte membrane composition and its behavior in the hyposmotic environment does not appear to occur in the diseased population of this study.

Discussion

The higher osmotic stability of erythrocytes in patients with sepsis and septic shock, evidenced by the lower values of A_{min}, H_0, H_{50} and H_{100}, as well as by the higher values of dX, in relation to the control group (Table 1), is a remarkable result of this study.

A good justification for this finding should be the existence of tendency to anemia or anemia in the diseased patients, as evidenced by the lower values of A_{max} (**Table 1** and **Figure 2**), hemoglobin levels, RBC counts, and values of hematocrit, MCH and/or MCHC in comparison to the control group (**Table 1**). Lower concentrations of hemoglobin will exert less osmotic pressure for water intake and higher volume for water occupation (Savitz, Sidel et al. 1964, Hladky and Rink 1978), making erythrocytes more capable of maintaining their integrity in the hyposmotic environment. The negative correlation observed between dX and A_{min}, not only in the diseased population but also in the control population and in several other clinical conditions (de Freitas, Marquez-Bernardes et al. 2014, Mascarenhas Netto Rde, Fabbri et al. 2014), is possibly a manifestation of this type of mechanism. Erythrocytes with less hemoglobin to release spontaneously in isosmotic conditions with blood (lower A_{min}) should withstand more against the decrease in saline concentration of the medium (greater dX).

It is possible that this anemia is associated with the existing inflammatory state. The observed negative correlations between blood hemoglobin levels and hematocrit values with C-reactive protein (CRP) (**Table 3**) support this idea.

Another possible cause of this anemia is the existence of malnutrition. Nutritional deficiencies of iron and protein are trivial causes of iron deficiency and/or

microcytic anemia. Indeed, the values of mean corpuscular volume (MCV) were lower, although without statistical significance, in the sepsis and septic shock groups when compared to control group. In addition, the strong correlation observed between MCV and MCH shows that this variation in erythrocyte volume occurs due to the influence of hemoglobin levels. In addition, the lowest values of total cholesterol (t-C), LDL-C (LDL-C) and HDL-C (HDL-C) observed in the sepsis and/or septic shock groups are quite suggestive of malnutrition.

In several studies the stability of erythrocytes has been associated with lipidemia (Bernardino Neto, de Avelar et al. 2013, de Freitas, Marquez-Bernardes et al. 2014, Aires Rodrigues de Freitas, Vieira da Costa et al. 2018) and in fact in this study there was an inverse correlation of H_{50} with blood levels of t-C and LDL-C in the control group, but not in the sick population (**Table 3**). This means that lipidemia, which helps to modulate the membrane cholesterol content, is not playing a role in the control of erythrocyte stability, which makes sense given the lower levels of t-C and LDL-C observed in the groups with sepsis and shock compared to the control group.

Another remarkable result of the present study is the existence of significantly higher RDW values in the sepsis and septic shock groups than in the control group (**Table 1**). This may be due, at least in part, to the fact that erythrocytes of people with sepsis or shock are less deformable than those of healthy volunteers (Moutzouri, Skoutelis et al. 2007). But why would the erythrocytes of the sick population be less deformable?

The deformability of erythrocytes may be influenced by the membrane cholesterol content. An excess of membrane cholesterol was associated with decreased erythrocyte deformability (Cooper, Arner et al. 1975, Muller, Ziegler et al. 1990). But this should not be the case of sepsis and septic shock, given the lower cholesterol levels observed here. Thus, the lower deformability of erythrocytes reported in sepsis and septic shock (Moutzouri, Skoutelis et al. 2007) should have other associations.

Deformability is a property related to osmotic membrane stability (Orbach, Zelig et al. 2017). In fact, significant correlations were observed between RDW and osmotic stability in other studies (Bernardino Neto, de Avelar et al. 2013, de Freitas, Marquez-Bernardes et al. 2014, Aires Rodrigues de Freitas, Vieira da Costa et al. 2018) and in the control group but not in the sick population of the present study (**Table 3**). Thus, it makes sense that the aforementioned associations comprise malnutrition and/or inflammatory aggression, leading to a decrease in the availability of iron in the body

and to the need to increase the life time of the erythrocyte, with an increase in the population of older erythrocytes and consequent decrease in deformability (**Figure 3**).

The existence of association between increase in RDW and elevation in the population of older erythrocytes had already been suggested (Patel, Patel et al. 2015). It is important to note that the existence of this predominance of older erythrocytes would occur not because of the acceleration of aging, but because of the organism's need to preserve its erythrocytes longer, especially in view of the existing inflammatory and malnutrition states. Indeed, lower iron availability in the body was associated with exacerbation of inflammation (Osterholm and Georgieff 2015). In addition, the negative correlation presented by blood levels of hemoglobin with C-reactive protein (CRP) (**Table 3**) does in fact allow the association of anemia with the inflammatory state in the present study.

Special attention should be given to the increase in the RDW values of the sick population, since an increase in the RDW has been associated with the prediction of worse clinical outcome in several diseases (Perlstein, Weuve et al. 2009, Patel, Semba et al. 2010, Bujak, Wasilewski et al. 2015, Vaya, Sarnago et al. 2015, Zurauskaite, Meier et al. 2018). The positive correlation observed between RDW and SAPS3 in the group of volunteers with sepsis of the present study also reveals the existence of an association with a higher chance of death with the highest values of RDW.

The present study presents some limitations that deserve to be highlighted. The first one is the lack of evaluation of glycated hemoglobin and bilirubin in all groups of this study, which would have been useful to estimate the erythrocyte life time, since high levels of glycated hemoglobin and low levels of bilirubin are suggestive of the existence of erythrocytes with longer life (Lewis and Gershow 1961, Cohen, Franco et al. 2008). The second is the lack of evaluation of oxidative variables, since the exacerbation of oxidative aggression and reduction of antioxidant defenses are factors capable of affecting the erythrocyte membrane structure and RDW values (Semba, Patel et al. 2010, Mohanty, Nagababu et al. 2014). A third limitation is the lack of information about the body's iron status, which could sustain the observed results, allowing analysis of the suggested association of iron availability with inflammation.

In spite of these limitations, the present study shows that: 1) erythrocytes from the study groups with sepsis and septic shock are more stable in hyposmotic medium than normal volunteers, 2) this increased osmotic stability of erythrocytes is associated

with anemia and inflammation, and 3) higher RDW values were associated with a higher chance of death.

References

Abbaspour, N., Hurrell, R., & Kelishadi, R. (2014). Review on iron and its importance for human health. *J Res Med Sci., 19*(2), 164–174.

Aires Rodrigues de Freitas, M., Vieira da Costa, A., Alves de Medeiros, L., da Silva Garrote Filho, M., Lemos Debs Diniz, A., & Penha-Silva, N. (2018). Are There Differences in the Anthropometric, Hemodynamic, Hematologic, and Biochemical Profiles between Late- and Early-Onset Preeclampsia? *Obstet Gynecol Int, 2018*, 9628726. https://doi.org/10.1155/2018/9628726

Bateman, R., Sharpe, M., Singer, M., & Ellis, C. (2017). The Effect of Sepsis on the Erythrocyte. *International Journal of Molecular Sciences, 18*(9), 1932. https://doi.org/10.3390/ijms18091932

Bateman, R. M., & Walley, K. R. (2005). Microvascular resuscitation as a therapeutic goal in severe sepsis. *Critical Care, 9*(Suppl 4), S27. https://doi.org/10.1186/cc3756

Bernardino Neto, M., de Avelar, E. B., Jr., Arantes, T. S., Jordao, I. A., da Costa Huss, J. C., de Souza, T. M., . . . Penha-Silva, N. (2013). Bivariate and multivariate analyses of the correlations between stability of the erythrocyte membrane, serum lipids and hematological variables. *Biorheology, 50*(5-6), 305-320. https://doi.org/10.3233/BIR-130641

Bone, R. C., Balk, R. A., Cerra, F. B., Dellinger, R. P., Fein, A. M., Knaus, W. A., . . . Sibbald, W. J. (1992). Definitions for Sepsis and Organ Failure and Guidelines for the Use of Innovative Therapies in Sepsis. *Chest, 101*(6), 1644–1655. https://doi.org/10.1378/chest.101.6.1644

Bosmann, M., & Ward, P. A. (2013). The inflammatory response in sepsis. *Trends Immunol, 34*(3), 129-136. https://doi.org/10.1016/j.it.2012.09.004

Bujak, K., Wasilewski, J., Osadnik, T., Jonczyk, S., Kolodziejska, A., Gierlotka, M., & Gasior, M. (2015). The Prognostic Role of Red Blood Cell Distribution Width in Coronary Artery Disease: A Review of the Pathophysiology. *Dis Markers, 2015*, 824624. https://doi.org/10.1155/2015/824624

Cherayil, B. J. (2015). Pathophysiology of Iron Homeostasis during Inflammatory States. *J Pediatr, 167*(4 Suppl), S15-19. https://doi.org/10.1016/j.jpeds.2015.07.015

Cohen, R. M., Franco, R. S., Khera, P. K., Smith, E. P., Lindsell, C. J., Ciraolo, P. J., . . . Joiner, C. H. (2008). Red cell life span heterogeneity in hematologically normal

people is sufficient to alter HbA1c. *Blood, 112*(10), 4284-4291. https://doi.org/10.1182/blood-2008-04-154112

Cooper, R. A., Arner, E. C., Wiley, J. S., & Shattil, S. J. (1975). Modification of red cell membrane structure by cholesterol-rich lipid dispersions. A model for the primary spur cell defect. *J Clin Invest, 55*(1), 115-126. https://doi.org/10.1172/JCI107901

de Freitas, M. V., Marquez-Bernardes, L. F., de Arvelos, L. R., Paraiso, L. F., Goncalves, E. O. A. F., Mascarenhas Netto Rde, C., . . . Penha-Silva, N. (2014). Influence of age on the correlations of hematological and biochemical variables with the stability of erythrocyte membrane in relation to sodium dodecyl sulfate. *Hematology, 19*(7), 424-430. https://doi.org/10.1179/1607845413Y.0000000145

Donadello, K., Piagnerelli, M., Reggiori, G., Gottin, L., Scolletta, S., Occhipinti, G., . . . Vincent, J. L. (2015). Reduced red blood cell deformability over time is associated with a poor outcome in septic patients. *Microvasc Res, 101*, 8-14. https://doi.org/10.1016/j.mvr.2015.05.001

Fleischmann, C., Scherag, A., Adhikari, N. K. J., Hartog, C. S., Tsaganos, T., Schlattmann, P., . . . Reinhart, K. (2016). Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations. *American Journal of Respiratory and Critical Care Medicine, 193*(3), 259-272. https://doi.org/10.1164/rccm.201504-0781OC

Hladky, S. B., & Rink, T. J. (1978). Osmotic behaviour of human red blood cells: an interpretation in terms of negative intracellular fluid pressure. *J Physiol, 274*, 437-446. https://doi.org/10.1113/jphysiol.1978.sp012158

Janz, D. R., Bastarache, J. A., Peterson, J. F., Sills, G., Wickersham, N., May, A. K., . . . Ware, L. B. (2013). Association Between Cell-Free Hemoglobin, Acetaminophen, and Mortality in Patients With Sepsis. *Critical Care Medicine, 41*(3), 784-790. https://doi.org/10.1097/CCM.0b013e3182741a54

Jo, Y. H., Kim, K., Lee, J. H., Kang, C., Kim, T., Park, H.-M., . . . Rhee, J. E. (2013). Red cell distribution width is a prognostic factor in severe sepsis and septic shock. *The American Journal of Emergency Medicine, 31*(3), 545-548. https://doi.org/10.1016/j.ajem.2012.10.017

Kosalka, K., Wachowska, E., & Slotwinski, R. (2017). Disorders of nutritional status in sepsis - facts and myths. *Prz Gastroenterol, 12*(2), 73-82. https://doi.org/10.5114/pg.2017.68165

Lewis, A. E., & Gershow, J. (1961). Estimation of erythrocyte life span from bilirubin clearance. *J Appl Physiol, 16*, 1140-1141. https://doi.org/10.1152/jappl.1961.16.6.1140

Machado, F. R., Assunção, M. S. C. d., Cavalcanti, A. B., Japiassú, A. M., Azevedo, L. C. P. d., & Oliveira, M. C. (2016). Getting a consensus: advantages and disadvantages of Sepsis 3 in the context of middle-income settings. *Revista Brasileira de Terapia Intensiva, 28*(4). https://doi.org/10.5935/0103-507X.20160068

Martin, S. L., Desai, S., Nanavati, R., Colah, R. B., Ghosh, K., & Mukherjee, M. B. (2018). Red cell distribution width and its association with mortality in neonatal sepsis. *J Matern Fetal Neonatal Med*, 1-6. https://doi.org/10.1080/14767058.2017.1421932

Mascarenhas Netto Rde, C., Fabbri, C., de Freitas, M. V., Bernardino Neto, M., Garrote-Filho, M. S., Lacerda, M. V., . . . Penha-Silva, N. (2014). Influence of Plasmodium vivax malaria on the relations between the osmotic stability of human erythrocyte membrane and hematological and biochemical variables. *Parasitol Res, 113*(3), 863-874. https://doi.org/10.1007/s00436-013-3717-4

Metnitz, P. G., Moreno, R. P., Almeida, E., Jordan, B., Bauer, P., Campos, R. A., . . . Investigators, S. (2005). SAPS 3--From evaluation of the patient to evaluation of the intensive care unit. Part 1: Objectives, methods and cohort description. *Intensive Care Med, 31*(10), 1336-1344. https://doi.org/10.1007/s00134-005-2762-6

Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P., & Malik, A. B. (2014). Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal, 20*(7), 1126-1167. https://doi.org/10.1089/ars.2012.5149

Mohanty, J. G., Nagababu, E., & Rifkind, J. M. (2014). Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging. *Front Physiol, 5*, 84. https://doi.org/10.3389/fphys.2014.00084

Moralez, G. M., Rabello, L., Lisboa, T. C., Lima, M., Hatum, R. M., De Marco, F. V. C., . . . Investigators, O. S. (2017). External validation of SAPS 3 and MPM0-III scores in 48,816 patients from 72 Brazilian ICUs. *Ann Intensive Care, 7*(1), 53. https://doi.org/10.1186/s13613-017-0276-3

Moreno, R. P., Metnitz, P. G., Almeida, E., Jordan, B., Bauer, P., Campos, R. A., . . . Investigators, S. (2005). SAPS 3--From evaluation of the patient to evaluation of

the intensive care unit. Part 2: Development of a prognostic model for hospital mortality at ICU admission. *Intensive Care Med, 31*(10), 1345-1355. https://doi.org/10.1007/s00134-005-2763-5

Moutzouri, A. G., Skoutelis, A. T., Gogos, C. A., Missirlis, Y. F., & Athanassiou, G. M. (2007). Red blood cell deformability in patients with sepsis: a marker for prognosis and monitoring of severity. *Clin Hemorheol Microcirc, 36*(4), 291-299.

Muller, S., Ziegler, O., Donner, M., Drouin, P., & Stoltz, J. F. (1990). Rheological properties and membrane fluidity of red blood cells and platelets in primary hyperlipoproteinemia. *Atherosclerosis, 83*(2-3), 231-237. https://doi.org/10.1016/0021-9150(90)90168-I

Orbach, A., Zelig, O., Yedgar, S., & Barshtein, G. (2017). Biophysical and Biochemical Markers of Red Blood Cell Fragility. *Transfus Med Hemother, 44*(3), 183-187. https://doi.org/10.1159/000452106

Osterholm, E. A., & Georgieff, M. K. (2015). Chronic inflammation and iron metabolism. *J Pediatr, 166*(6), 1351-1357 e1351. https://doi.org/10.1016/j.jpeds.2015.01.017

Patel, H. H., Patel, H. R., & Higgins, J. M. (2015). Modulation of red blood cell population dynamics is a fundamental homeostatic response to disease. *Am J Hematol, 90*(5), 422-428. https://doi.org/10.1002/ajh.23982

Patel, K. V., Mohanty, J. G., Kanapuru, B., Hesdorffer, C., Ershler, W. B., & Rifkind, J. M. (2013). Association of the red cell distribution width with red blood cell deformability. *Adv Exp Med Biol, 765*, 211-216. https://doi.org/10.1007/978-1-4614-4989-8_29

Patel, K. V., Semba, R. D., Ferrucci, L., Newman, A. B., Fried, L. P., Wallace, R. B., . . . Guralnik, J. M. (2010). Red cell distribution width and mortality in older adults: a meta-analysis. *J Gerontol A Biol Sci Med Sci, 65*(3), 258-265. https://doi.org/10.1093/gerona/glp163

Penha-Silva, N., Firmino, C. B., de Freitas Reis, F. G., da Costa Huss, J. C., de Souza, T. M., de Freitas, M. V., & Netto, R. C. (2007). Influence of age on the stability of human erythrocyte membranes. *Mech Ageing Dev, 128*(7-8), 444-449. https://doi.org/10.1016/j.mad.2007.06.007

Perlstein, T. S., Weuve, J., Pfeffer, M. A., & Beckman, J. A. (2009). Red blood cell distribution width and mortality risk in a community-based prospective cohort. *Arch Intern Med, 169*(6), 588-594. https://doi.org/10.1001/archinternmed.2009.55

Piagnerelli, M., Boudjeltia, K. Z., Vanhaeverbeek, M., & Vincent, J. L. (2003). Red blood cell rheology in sepsis. *Intensive Care Med, 29*(7), 1052-1061. https://doi.org/10.1007/s00134-003-1783-2

Savitz, D., Sidel, V. W., & Solomon, A. K. (1964). Osmotic Properties of Human Red Cells. *J Gen Physiol., 48*(1), 79–94. https://doi.org/10.1085/jgp.48.1.79

Semba, R. D., Patel, K. V., Ferrucci, L., Sun, K., Roy, C. N., Guralnik, J. M., & Fried, L. P. (2010). Serum antioxidants and inflammation predict red cell distribution width in older women: the Women's Health and Aging Study I. *Clin Nutr, 29*(5), 600-604. https://doi.org/10.1016/j.clnu.2010.03.001

Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., Bauer, M., . . . Angus, D. C. (2016). The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *Jama, 315*(8), 801. https://doi.org/10.1001/jama.2016.0287

Straat, M., van Bruggen, R., de Korte, D., & Juffermans, N. P. (2012). Red blood cell clearance in inflammation. *Transfus Med Hemother, 39*(5), 353-361. https://doi.org/10.1159/000342229.

Sutera, S. P., Gardner, R. A., Boylan, C. W., Carroll, G. L., Chang, K. C., Marvel, J. S., . . . Williamson, J. R. (1985). Age-related changes in deformability of human erythrocytes. *Blood, 65*(2), 275-282. https://doi.org/10.1182/blood.V65.2.275.275

Vaya, A., Sarnago, A., Fuster, O., Alis, R., & Romagnoli, M. (2015). Influence of inflammatory and lipidic parameters on red blood cell distribution width in a healthy population. *Clin Hemorheol Microcirc, 59*(4), 379-385. https://doi.org/10.3233/CH-141862

Zurauskaite, G., Meier, M., Voegeli, A., Koch, D., Haubitz, S., Kutz, A., . . . Schuetz, P. (2018). Biological pathways underlying the association of red cell distribution width and adverse clinical outcome: Results of a prospective cohort study. *PLoS One, 13*(1), e0191280. https://doi.org/10.1371/journal.pone.0191280

Table 1. Osmotic stability and hematologic and lipid profiles of study populations

Parameters	Control (N=49)	Sepsis (N=25)	Septic shock (N=25)
Age (years)	48.27 ± 14.5	48.88 ± 20.57	47.24 ± 18.37
A_{min} (10^{-2}) (abs)	1.35 (0.45-2.07)	1.03 (0.51-1.65)	0.59 (0.12-1.46)
A_{max} (abs)	1.32 (1.2-1.42) [a,b]	0.7 (0.62-0.87) [a]	0.75 (0.65-0.82) [b]
H_0 (g/dL)	0.48 (0.46-0.49) [a,b]	0.43 (0.41-0.47) [a]	0.43 (0.42-0.45) [b]
H_{50} (g/dL)	0.45 (0.43-0.46) [a,b]	0.39 (0.34-0.42) [a]	0.39 (0.35-0.42) [b]
H_{100} (g/dL)	0.41 (0.4-0.43) [a,b]	0.33 (0.29-0.38) [a]	0.34 (0.31-0.39) [b]
dX (10^{-2}) (g/dL)	1.44 (1.27-1.94) [a,b]	3.09 (0.99-3.49) [a]	2.22 (1.25-2.99) [b]
Hb (g/dL)	15.1 (14.4-15.7) [a,b]	9.25 (8.4-10.7) [a]	8.8 (8-9.8) [b]
Ht (%)	45 (42.2-47) [a,b]	27.9 (25.85-30.85) [a]	27.1 (24.4-30) [b]
RBC (millions/mm^3)	4.86 (4.59-5.2) [a,b]	3.2 (3-3.37) [a]	2.97 (2.89-3.24) [b]
MCV (fL)	92.1 (86.7-95.6)	87.5 (84.2-93.5)	86.5 (84.2-91.4)
MCH (pg)	31 (28.9-32.5) [b]	29.35 (28.4-29.9)	28.9 (27.5-30.2) [b]
MCHC (g/dL)	33.69 ± 0.73 [b]	33.11 ± 1.61	32.58 ± 1.09 [b]
RDW (%)	13.19 ± 0.76 [a,b]	15.32 ± 1.72 [a]	14.47 ± 1.53 [b]
TGC (mg/dL)	121.09 (89.65-151.85)	174.3 (104.48-242.75)	144 (93-230)
t-C (mg/dL)	184.2 ± 36.74 [a,b]	141.45 ± 46.45 [a]	131.5 ± 33.65 [b]
LDL-C (mg/dL)	116.46 ± 33.1 [a]	84.29 ± 50.57 [a]	95.79 ± 28.65
VLDL-C (mg/dL)	24.4 (17.93-30.35)	34.9 (20.6-48.5)	28.8 (18.3-46)
HDL -C (mg/dL)	40.7 (34-48.4) [a,b]	20.7 (13-31.2) [a]	26.4 (15.4-33) [b]

[a,b] Statistically significant differences ($p < 0.05$) are indicated by pairs of the same letter associated with the values of the parameters studied, expressed as mean ± standard deviation or median (25%-75% percentile) for parameters with values normally and not normally distributed, respectively.

Abbreviations: A_{min}, absorbance at 540 nm associated with initial hemolysis under isosmotic conditions with blood; A_{max}, absorbance at 540 nm associated with total hemolysis; H_0, concentration of NaCl capable of initiating *in vitro* hemolysis; H_{50}, NaCl concentration capable of promoting 50% hemolysis *in vitro*; H_{100} concentration of NaCl capable of promoting total hemolysis *in vitro*; dX, variation of NaCl concentration responsible for ¼ of total hemolysis *in vitro*; Hb, hemoglobin; Ht, hematocrit; RBC, erythrocytes; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red-cell distribution width; TGC, triglycerides; t-C, total cholesterol; LDL-C, low- density lipoprotein cholesterol; VLDL-C, very low density cholesterol; HDL-C, high-density lipoprotein cholesterol.

Table 2. Parameters evaluated in individuals with sepsis and septic shock who participated in the study *

Parameters	Sepsis (N=25)	Septic Shock (N=25)	p
Plt (10^6/ mm^3)	267 (430-155)	212 (357-137)	0.3795
WBC (10^6/ mm^3)	13.51 (22.85-9.54)	14 (20.2-9.9)	0.8315
CRP (U/L)	6.1 (10.7-4.31)	13 (17.15-8.01)	0.1419
Lactate (mmol/L)	1.7 (2-1.2)	1.7 (2.2-1.5)	0.4045
Urea (mg/dL)	53.85 (124.5-37.7)	49 (78-40)	0.7612
Crn (mg/dL)	0.99 (2.62-0.63)	0.83 (1.9-0.6)	0.5090
Na^+ (mEq/L)	142 (144-137)	141 (146-136)	0.9149
K^+ (mEq/L)	4.55 ± 0.96	4.17 ± 0.92	0.1801
pH	7.41 (7.44-7.33)	7.39 (7.43-7.35)	0.6623
pCO_2 (mmHg)	38.5 (48.7-34.5)	36.7 (44-35.5)	0.8445
pO_2 (mmHg)	84.17 ± 19.04	95.94 ± 35.49	0.5090
HCO_3^- (mmol/L)	23.89 ± 4.18	23.43 ± 3.96	0.4389
$SatO_2$ (%)	95.4 (97-94.2)	96.3 (98-94.1)	0.2694
BE (mmol/L)	-0.61 ± 3.77	-1.58 ± 4.26	0.2353
SAPS3	61 (69-56)	61 (75-55)	0.8842

* Values expressed as mean ± standard deviation or median (25%-75% percentile) for variables with normal or non-normal distribution, respectively.

Abbreviations: Plt, platelets; WBC, leukocytes; CRP, C-reactive protein; Crn, creatinine; Na^+, sodium; K^+, potassium; pH, hydrogen potential; pCO_2, partial pressure of carbon dioxide; pO_2, partial pressure of oxygen; HCO_3^-, bicarbonate; $SatO_2$, oxygen saturation; BE, base excess; SAPS3, Simplified Acute Physiology Score 3.

Table 3. Spearman correlations involving osmotic stability and hematologic and biochemical variables *

Main	Related	Control Group		Sepsis Group		Septic Shock Group	
		rho	p	rho	p	rho	p
$1/H_{50}$	dX	0.050	0.736	0.408	0.093	0.661	0.002
	A_{max}	-0.022	0.881	0.542	0.020	0.119	0.627
	H_0	-0.868	< 0.001	-0.775	< 0.001	-0.800	< 0.001
	H_{100}	-0.908	< 0.001	-0.905	< 0.001	-0.951	< 0.001
	RBC	-0.136	0.357	-0.580	0.030	0.134	0.595
	t-C	0.289	0.046	-0.297	0.405	0.309	0.385
	LDL-C	0.349	0.015	-0.261	0.467	-0.033	0.932
dX	A_{min}	-0.226	0.118	-0.564	0.018	-0.718	0.001
	H_0	0.335	0.020	0.117	0.645	-0.132	0.591
	H_{100}	-0.374	0.009	-0.719	0.001	-0.828	< 0.001
	RBC	0.340	0.017	-0.207	0.477	0.054	0.832
	RDW	0.472	0.001	-0.288	0.364	0.264	0.290
	MCH	-0.334	0.019	-0.112	0.729	0.136	0.590
Amin	H_{100}	0.087	0.555	0.321	0.209	0.533	0.019
H_{100}	Ht	0.161	0.273	0.534	0.027	0.193	0.443
	t-C	-0.328	0.023	0.103	0.777	-0.248	0.489
	TGC	0.323	0.025	0.220	0.471	0.047	0.879
	LDL-C	-0.385	0.007	0.091	0.803	-0.033	0.932
	VLDL-C	0.292	0.044	0.109	0.749	-0.479	0.162
	HDL-C	-0.311	0.032	0.067	0.855	0.150	0.700
CRP	Amax			-0.429	0.289	-0.673	0.033
	H_0			0.738	0.037	0.067	0.855
	RBC			-0.303	0.364	-0.588	0.074
	Hb			-0.361	0.226	-0.645	0.032
	Ht			-0.313	0.297	-0.588	0.057
	MCHC			0.273	0.391	-0.560	0.073
	t-C			-0.700	0.036	0.551	0.257
	LDL-C			-0.733	0.025	0.600	0.285
	satO2			-0.552	0.063	0.312	0.324
RDW	RBC	0.397	0.005	0.474	0.047	-0.135	0.559
	Ht	-0.093	0.527	0.602	0.008	-0.105	0.649
	MCH	-0.666	< 0.001	-0.149	0.554	0.128	0.582
	MCV	-0.621	< 0.001	0.184	0.465	0.206	0.369
	MCHC	-0.125	0.392	-0.594	0.009	-0.202	0.380
	t-C	0.303	0.034	0.311	0.259	0.107	0.741
	SAPS3			0.756	< 0.001	0.209	0.362
RBC	Hb	0.585	< 0.001	0.816	< 0.001	0.647	0.002
	Ht	0.578	< 0.001	0.908	< 0.001	0.607	0.004
	MCH	-0.613	< 0.001	-0.337	0.171	-0.284	0.212
	MCV	-0.607	< 0.001	0.111	0.632	-0.298	0.190
	MCHC	-0.009	0.952	-0.580	0.006	0.012	0.959

* Gray shading indicates statistically significant correlations ($p < 0.05$).

Abbreviations: A_{min}, absorbance at 540 nm associated with hemolysis under isosmotic conditions with blood; A_{max}, absorbance at 540 nm associated with total hemolysis; H_0, concentration of NaCl capable of initiating *in vitro* hemolysis; H_{100} concentration of NaCl capable of promoting total hemolysis; $1/H_{50}$, inverse of NaCl concentration capable of

promoting 50% hemolysis; dX, variation in NaCl concentration responsible for ¼ of the total hemolysis; Hb, hemoglobin; Ht, hematocrit; RBC, erythrocytes; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; TGC, triglycerides; t-C, total cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CRP, C-Reactive Protein; SAP3, Simplified Acute Physiology Score 3

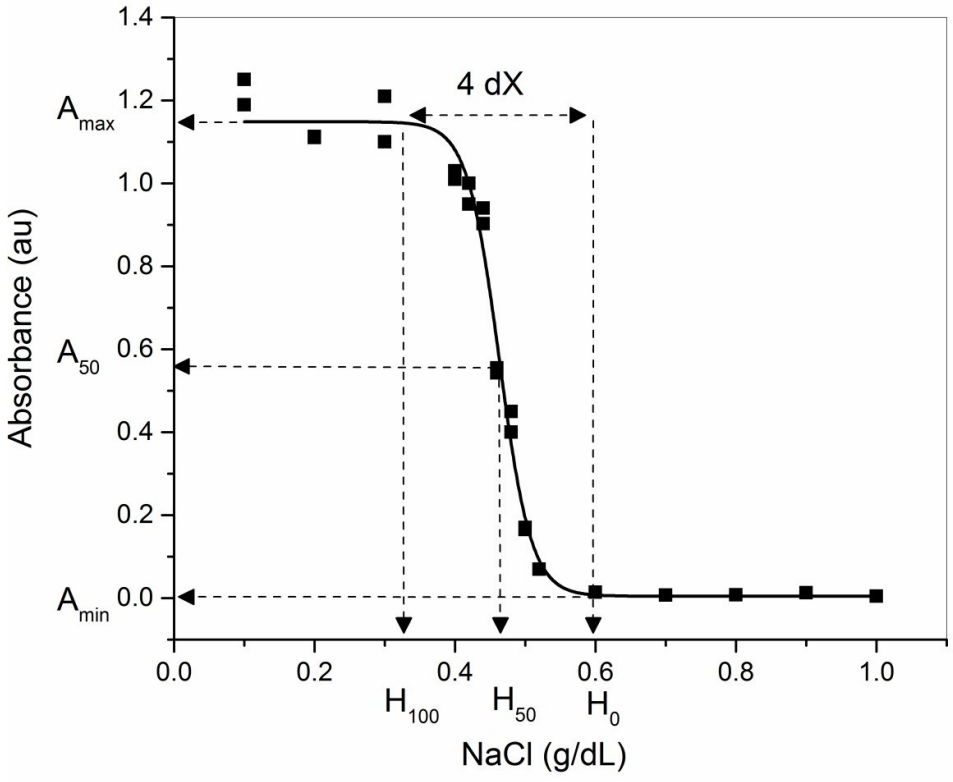

Figure 1. Typical osmotic stability curve of one of the volunteers with sepsis of the present study. A_{min} and A_{max} represent the minimum and maximum values of absorbance promoted by hemoglobin released in lysis in medium with higher and lower saline concentrations, respectively. H_{50} is the concentration of NaCl capable of promoting 50% hemolysis. dX is the change in NaCl concentration which promotes ¼ of the total lysis of erythrocytes. H_0 and H_{100} are the saline concentrations where *in vitro* hemolysis begins and ends, respectively.

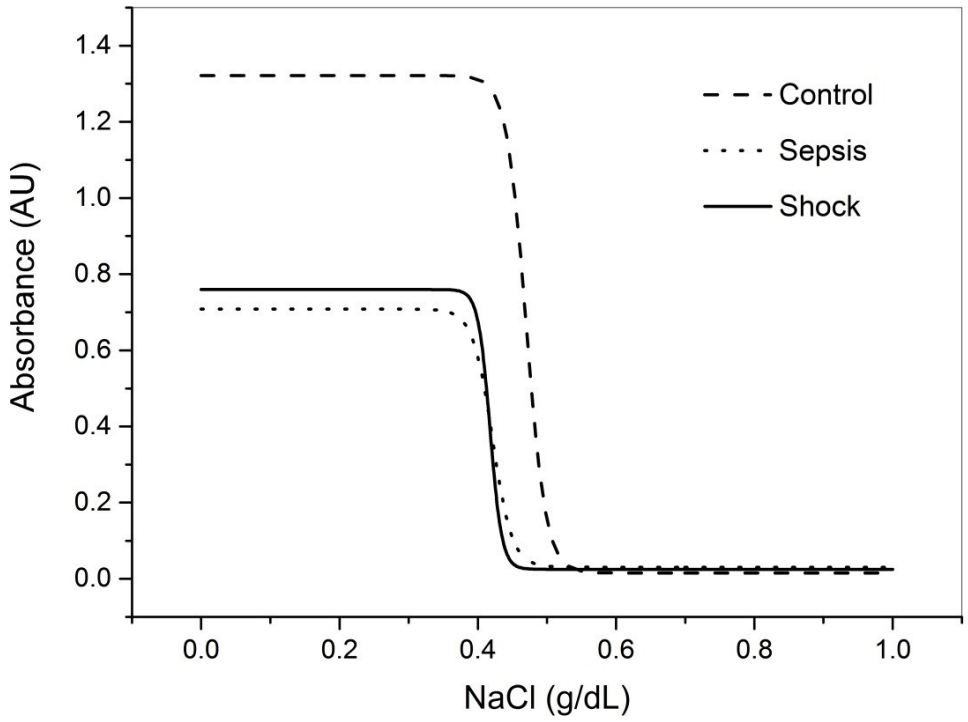

Figure 2. Osmotic fragility curves of volunteers from the control, sepsis and septic shock groups, respectively.

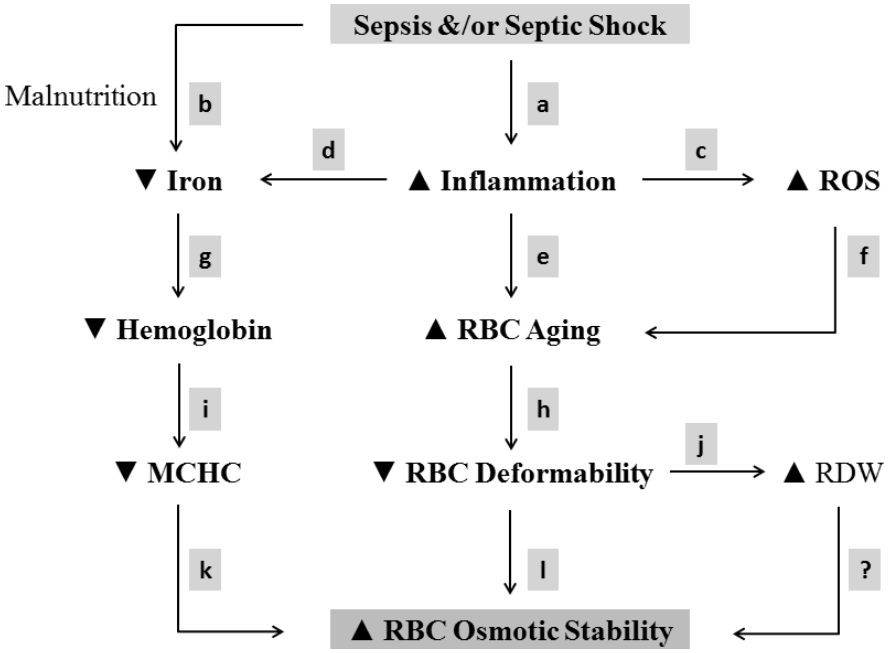

Figure 3. Possible associations, compiled from the literature and/or from results of this study, between sepsis and/or septic shock and osmotic stability of erythrocytes: a. (Bosmann and Ward 2013), b. (Kosalka, Wachowska et al. 2017), c. (Mittal, Siddiqui et al. 2014), d. (Cherayil 2015), e. (Straat, van Bruggen et al. 2012), f. (Mohanty, Nagababu et al. 2014), g. (Abbaspour, Hurrell et al. 2014), h. (Sutera, Gardner et al. 1985), i. (Piagnerelli, Boudjeltia et al. 2003), j. (Orbach, Zelig et al. 2017), k. (Hladky and Rink 1978), l. (Patel, Mohanty et al. 2013).

CAPÍTULO 3

Osmotic stability of erythrocyte membrane in pregnancy hypertensive disorders

Osmotic stability of erythrocyte membrane in pregnancy hypertensive disorders

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Abstract

Background: Hypertensive disorders of pregnancy are complex processes associated with placental changes and include elevations of liver enzymes, changes in lipid profile and erythrocyte membrane. This study aimed to evaluate the existence of changes in erythrocyte membrane stability in hypertensive diseases of pregnancy.

Materials and methods: A population of 32 pregnant women was stratified into four groups: control, gestational hypertension, preeclampsia superimposed on chronic hypertension and severe preeclampsia. The osmotic stability of the erythrocyte membrane was represented by minimum (A_{min}) and maximum (A_{max}) values of the free hemoglobin absorbance, the inverse of the NaCl concentration that can promote 50% hemolysis ($1/H_{50}$) and the variation in the concentration of NaCl required to cause 100% lysis (dX).

Results: A significant increase ($p<0.05$) in $1/H_{50}$ was observed in the group with gestational hypertension, although in preeclampsia superimposed on chronic hypertension occurred a borderline reduction ($0.05<p<0.10$) in this variable. Furthermore, a significant decrease ($p <0.01$) was observed in the A_{min} values among pregnant women with severe preeclampsia. **Conclusions:** All of gestation hypertensive diseases considered in this study was associated with some kind of change in the erythrocyte membrane stability.

Keywords: Hypertension. Preeclampsia. Erythrocytes. Stability.

Introduction

Hypertensive disorders of pregnancy are a common problem of high maternal and fetal mortality. In pregnancy hypertension is associated with placental changes and, in more severe cases, there is change in the profile of liver enzymes, hemolysis and reduction in the number of platelets (Carvalho, Faundes et al. 1997, Leeman and Fontaine 2008).

Hypertensive disorders of pregnancy are classified into four groups: pre- eclampsia, chronic hypertension (of any etiology), preeclampsia superimposed on chronic hypertension and gestational hypertension (2013).

In mild preeclampsia there is increase in pressure accompanied by proteinuria (excretion of 0.3 g protein in the 24 hours urine or intensity ≥ 1+ on urine dipsticks). Severe preeclampsia is diagnosed if the systolic blood pressure is ≥ 160 mmHg and the diastolic blood pressure is ≥ 110 mmHg, when measured in patients at rest at four-hour intervals, or when hypertension is associated with thrombocytopenia (platelet count < $10^3/mm^3$), worsening in the hepatic function (elevation of blood levels of hepatic enzymes more than twice the upper reference range limit), worsening in the renal function (creatinine > 1.1 mg/dL), pulmonary edema and signs of hypertensive encephalopathy (2013).

Chronic hypertension is characterized by elevated blood pressure before pregnancy or before completing 20 weeks of gestation. In preeclampsia superimposed on chronic hypertension occurs worsening in the arterial blood pressure. In preeclampsia the change in pressure is accompanied by proteinuria. Gestational hypertension is characterized by an isolated change in the blood pressure (2013, Magee, Pels et al. 2014).

Hypertension was associated with changes in the erythrocyte membrane, such as changes in the behavior of its sodium channels and lithium (Canessa, Adragna et al. 1980, Tsuda, Minatogawa et al. 1987) and also in its lipid composition, due to changes in the blood lipid levels, such as decrease in HDL-C and increase in VLDL- and LDL-C (Zicha, Kunes et al. 1999).

The erythrocyte membrane's ability to change its shape and resist the force determined by blood flow and by friction with the vessel wall (Mohandas and Evans 1994) is mainly due to the cytoskeleton proteins and to its proper lipid composition (Mohandas and Evans 1994).

The erythrocyte membrane's ability to preserve its structure before any kind of situation is called stability (Cunha, Arvelos et al. 2007, Penha-Silva, Firmino et al. 2007, Penha-Silva, Arvelos et al. 2008). The erythrocyte membrane stability can be influenced by intrinsic factors of the membrane and the cell, but also by extrinsic factors, which include the composition and properties of the medium and the wide range of factors that can affect metabolism. The proper dynamics of erythropoiesis, which will affect qualitatively and quantitatively erythrocytes, is influenced by several key nutritional factors, such as iron, amino acids, folate, cobalamin, pyridoxine and lipid membrane.

Furthermore, the lipid composition of the membrane of mature erythrocytes is an intrinsic condition that can be influenced by the nature and levels of circulating lipids (Pacetti, Gagliardi et al. 2016). This influence is related to the dynamics of the exchanges that take place between the membrane of mature circulating erythrocyte and plasma lipoproteins. The circulating erythrocytes is capable of receiving cholesterol from LDL (Cooper, Leslie et al. 1978), which is an important physiological mechanism for regulating the fluidity and stability that cell membrane. That is why increase in blood cholesterol levels is associated with increase in counts not only of erythrocytes, but also of other blood cells such as platelets, at least in certain segments of the population (Fessler, Rose et al. 2013). However, an excessive increase in LDL- cholesterol (LDL-C) leads to excessive incorporation of cholesterol in the erythrocyte membrane, with a decrease in the fluidity and stability of the RBC membrane. That is why a greater increase in blood cholesterol is associated with the decrease of erythrocytes and platelets counts in other segments of the population (Fessler, Rose et al. 2013). If the blood cholesterol levels reach the pathological levels present in individuals with familial hypercholesterolemia, excessive incorporation of cholesterol in the RBC membrane will cause the so-called spur-cell anemia (Cooper 1969).

Thus, it is not without sense that the variables usually determined in erythrogram are affected by the cholesterol levels in the blood (Bernardino Neto, de Avelar et al. 2013, de Arvelos, Rocha et al. 2013, de Freitas, Marquez-Bernardes et al. 2014). Indeed, an increase in the RDW was associated with high cholesterol content in the erythrocyte membrane (Tziakas, Chalikias et al. 2012). Certainly this is the reason why the erythrocyte membrane stability was associated with the blood cholesterol levels and the RDW (de Arvelos, Rocha et al. 2013, de Freitas, Marquez-Bernardes et al. 2014, Netto, Fabbri et al. 2014). This is very important and can have significant clinical

implications, since the RDW has been associated with the prediction of a wide range of degenerative diseases (Patel, Ferrucci et al. 2009, Patel, Semba et al. 2010, Tziakas, Chalikias et al. 2010, Zalawadiya, Zmily et al. 2011, Malandrino, Wu et al. 2012, Tziakas, Chalikias et al. 2012, Zalawadiya, Veeranna et al. 2012, Patel, Mohanty et al. 2013), because it is able to reflect the harmful implications of dyslipidemia in the etiology of endothelial and gas exchange dysfunctions, what puts the erythrocyte as a protagonist in the etiology of the vascular degenerative disorders and their pathophysiological complications.

The membrane stability variables have shown to be promising to reflect changes in the lipid profile and erythrocyte indices associated with aging (Penha-Silva, Firmino et al. 2007, de Freitas, Marquez-Bernardes et al. 2014), energy restriction imposed by bariatric surgery (de Arvelos, Rocha et al. 2013), physical activity (Paraiso, de Freitas et al. 2014) and even infectious diseases as malaria (Netto, Fabbri et al. 2014).

As hypertensive pregnancy diseases are generally associated with changes in serum lipids and in the turnover rate of erythrocytes, it is possible that those diseases are also associated with changes in the erythrocyte membrane stability. The purpose of this study is to investigate this hypothesis.

Material and methods

Population

The study project was approved by the Ethics Committee of the Federal University of Uberlândia (3008/2014). The study included 32 pregnant patients who sought the Clinical Hospital of the Federal University of Uberlândia, Minas Gerais, Brazil, from July 2015 to April 2016. Pregnant patients who showed no changes in blood pressure, blood count and their biochemical tests were included in the control (C) group (n = 9). Patients who had an elevation in arterial blood pressure after completing 20 weeks of gestation were included in the gestational hypertension (GH) group (n = 7). Patients who already had a history of hypertension before pregnancy and developed a more severe pressure frame associated with proteinuria were included in preeclampsia superimposed on chronic hypertension (PSCH) group ($n=7$). The patients with blood pressure ≥ 140 x 90 mmHg after 20 weeks of pregnancy associated with proteinuria or hypertension associated with thrombocytopenia, hepatic, renal and cerebrovascular disorders or blood pressure ≥ 160 x 110 mmHg were included in the severe

preeclampsia (SP) group (*n=9*). The study excluded pregnant women who have other diseases and/or were users of tobacco or abuse drugs.

Collection of blood samples

Blood samples were collected by venipuncture into tubes containing K_3EDTA (for hematologic analysis and determining the stability of erythrocytes) and in tubes without anticoagulant (for biochemical analysis) (Vacutainer; Becton Dickinson, Juiz de Fora, Brazil).

Determination of the osmotic stability of human erythrocytes

The determination of the osmotic stability of erythrocytes was performed as described by Penha-Silva et al (Penha-Silva, Firmino et al. 2007). Initially, duplicate sets of test tubes containing 1 ml of 0.1-1.5 g/dL NaCl solutions (Labsynth, Diadema, SP, Brazil) were preincubated at 37 °C for 10 min. After addition of 10 µL of total blood and gentle agitation, the tubes were incubated at 37 °C for 30 min. After centrifugation at 1500 x *g* for 10 min, the supernatants were used to evaluate the optical density at 540 nm (A_{540}) in a UV-VIS spectrophotometer (Shimadzu™, model UV1650TC, Japan). The graphs of A_{540} as a function of the NaCl concentration (X) were fitted by non-linear regression in accordance with the Boltzmann equation:

$$A_{540} = \frac{A_{max} - A_{min}}{1 + e^{(X - H_{50})/dX}} + A_{min} \quad (1),$$

where A_{max} and A_{min} represent respectively the minimum and maximum plateaus of A_{540}, H_{50} is the NaCl concentration capable of promoting 50% hemolysis, and dX is the variation in concentration of NaCl responsible for 100% hemolysis.

Determination of haematological and biochemical parameters

The hematological parameters were obtained using an automated system (Sysmex K4500; Sysmex Corporation™, Mundelein, IL, USA). These parameters include erythrocytes (RBC), platelets (Plt) reticulocytes counts and the values of hematocrit (Ht), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV) and prothrombin time (PT).

The biochemical parameters were measured using an automated analyzer (Architect c8000, IL, USA). These parameters include triglycerides (TGC), total

cholesterol (t-C), very-low density lipoprotein cholesterol (VLDL-C), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), creatinine (C), uric acid (UA), sodium (Na^+), potassium (K^+), indirect bilirubin (iB), total bilirubin (tB), serum iron (Fe) and human serum albumin (HSA).

The reference values (women over 16 years of age) were: RBC, 4.3-5.0 x $10^6/mm^3$; Hb, 12.0–16.0 g/dL; Ht, 35.6–48.6%; MCV, 82–98 fL; MCH, 27–31 pg; MCHC, 32.9–36%; RDW, 12-15%; RI, 0.5-2.5%; Plt, 150-450 x $10^3/mm^3$; MPV, 7-10 fL; t-C, <200 (optimum) and ≥240 mg/dL (high); TGC, <150 (optimum) and >201 mg/dL (high); VLDL-C, up to 40 mg/dL; LDL-C, <100 (good) and >160 mg/dL (high); HDL-C, <45 (low) and >65 mg/dL (ideal); AST, 5-34 IU/L; ALT, 0-55 IU/L; LDH, 100-190 IU/L; AP, 35-104 IU/L; urea, 10-45 mg/dL; creatinine, 0.7-1.2 mg/dL; uric acid, 2.4-6.0 mg/dL; Na^+, 135-145 mEq/L; K^+, 3.7-5.6 mEq/L; iB, 0.2-0.8 mg/dL; tB, < 1.2 mg/dL; Fe, 65-175 μg/dL; ferritin, 6-159 ng/mL; and HAS, 3.5-5.2 g/dL.

Statistical analyzes

The normality of the data was assessed by the Shapiro-Wilk test. Most variables were not normally distributed. Median and interquartile range were used to represent the measurements of biochemical, hematological and membrane stability variables. The comparison between groups was performed using the Mann-Whitney test. Differences associated with p values < 0.05 were considered statistically significant. Differences with *p* values between 0.05 and 0.10 were considered borderline. All analyzes were performed using the software Origin 8.5 Professional (Microcal, Northampton, MA, USA) and/or GraphPad Prism 6.01 (La Jolla, CA, USA).

Results

A typical curve used in determining the erythrocyte membrane stability variables is shown in Figure 1. A condition of isotonicity with the blood occurs in the right region of the curve, where there is a plateau that defines the variable A_{min}, which represents the amount of basal hemolysis present in the sample of blood taken from each volunteer. A decrease in the medium tonicity is associated with an increase in absorbance at 540 nm, due to increase in the amount of hemoglobin that is released into solution by the

hemolysis process, which defines a sigmoidal curve whose upper plateau sets the variable A_{max}. This curve passes through an intermediate point that defines the variable H_{50}, which represents the concentration of NaCl required to promote 50% hemolysis. The curve also defines the variable dX, which represents the change in salt concentration needed to promote 100% hemolysis. The variable dX has a same direction relationship with the osmotic stability of erythrocytes, but the H_{50} variable has an opposite direction relationship and, therefore, it was used as a $1/H_{50}$, so that an increase in the values of both dX and $1/H_{50}$ could indicate increased osmotic stability of erythrocytes.

Table 1 shows the baseline characteristics of the four groups that constitute the study population. The variables values were presented as median ± interquartile range. The groups were compared with respect to all variables using the Mann-Whitney test.

The group with gestational hypertension (GH) showed a significant increase in membrane stability parameter $1/H_{50}$ compared with the group with preeclampsia superimposed on chronic hypertension (PSCH) (Figure 2). When compared to control (C), the GH group had a significant increase in the creatinine levels and a borderline increase in the VLDL-C levels. A borderline decrease in LDH was also observed in the group CH when compared to the control group (Table 1).

The PSCH group showed a decrease in the parameter $1/H_{50}$ compared to the group of women with severe preeclampsia (SP) (Figure 2). When compared with the control group, the SP group showed significant elevations in ALT and creatinine and borderline elevations in ferritin. A borderline decrease in LDH was also observed in the SP group compared to the control group (Table 1).

The SP group showed a decrease in membrane stability parameter A_{min} when compared to the groups GH and C (Figure 2). When compared with the control group, the SP group showed significant elevations of creatinine, uric acid, ferritin and ALT and a borderline elevation of AST, as well as a significant decrease in the levels of VLDL-C. The blood levels of AST and LDH of the SP group were significantly higher and the levels of VLDL-C were significantly lower in relation to the GH group. Blood levels of ALT and uric acid in the SP group also showed borderline elevations when compared to group with chronic hypertension. Furthermore, the blood levels of creatinine, uric acid and LDH of the SP group were significantly higher when compared to the PSCH group (Table 1).

Discussion

In the hypertensive diseases erythrocytes were subjected to aggressions of high blood pressure and friction with the vascular endothelium, which alter the shape of these cells and raise the rate of hemolysis (Hofmeyr and Belfort 2009, Hernandez Hernandez, Villasenor et al. 2015).

Indeed, in the group of pregnant women with PSCH the parameter $1/H_{50}$ was lower when compared to the SP group, but not in relation to the control group. However, the preservation of the value of $1/H_{50}$ in patients with gestational hypertension compared to the control group means that these erythrocytes remained osmotically resistant (Figure 2).

This preservation of the osmotic stability of erythrocytes could be due to an intrinsic factor, as the increase in the turnover of these cells. In fact, the turnover of red blood cells rises during pregnancy (Lurie and Mamet 2000) and particularly in preeclampsia (Troeger, Holzgreve et al. 2006).

On the other hand, it is possible that the preservation of the erythrocytes stability may be due to extrinsic factors such as an increase in the cholesterol exchange rate. A larger amount of circulating nHDL-C would enhance the availability of cholesterol for the erythrocyte membrane, with increase in its stability (Chabanel, Flamm et al. 1983, Bernardino Neto, de Avelar et al. 2013, de Arvelos, Rocha et al. 2013, de Freitas, Marquez-Bernardes et al. 2014). Thus, the erythrocyte would become more resistant, which would imply higher values of $1/H_{50}$, as occurred in patients with gestational hypertension. Indeed, higher VLDL-C values were observed in patients with gestational hypertension compared to the control group of this study (Table 1). In addition, higher levels of triglycerides, VLDL- and LDL-C are common in hypertensive patients (Bagdade, Buchanan et al. 1995), which could affect the membrane of erythrocytes (Zicha, Kunes et al. 1999). The existence of more stable erythrocyte would mean that the *in vivo* hemolysis would not be contributing to elevation of the LDH levels, since red blood cells are an important source of the LDH found in plasma (Catanzarite, Steinberg et al. 1995, Vazquez-Rodriguez, Rios-Gutierrez et al. 2016). Indeed, there was a borderline decrease in LDH levels in the group of pregnant women with gestational hypertension.

The significant decrease of Amin values in pregnant women with severe preeclampsia in the control group and borderline in the group with gestational

hypertension (Figure 2) indicates that the red blood cells of pregnant women with SP had increased membrane stability. This variable is proportional to the rate of hemolysis already present even in a condition which is isotonic with blood. This means that the blood collected from pregnant women with preeclampsia had more stable erythrocytes even in the *in vivo* conditions of tonicity.

In light of the fact that, compared to GH and PSCH groups, there was an increase in LDH levels in the SP group, the improved stability of erythrocytes in the SP group may appear contradictory if *in vivo* hemolysis was the main source of this biomarker. However, since the AST and ALT levels were also increased in this group, this indicates that the major source of these biomarkers should not be erythrocytes but more probably other cells such as the hepatocytes. In fact, the liver is a known target of hypertensive aggression present in preeclampsia. Further evidence that the liver is the source of these enzymes is the decrease in the levels of VLDL-C observed in this group, since the hepatic impairment harm lipogenesis, esterification and assembly of VLDL. The decrease in HSA levels in this group is further evidence of hepatic dysfunction.

Thus, the increased stability of erythrocytes of pregnant women with pre-eclampsia should not be due to environmental factors, but rather from a factor that is intrinsically associated with erythropoiesis. This makes sense, since elevation of erythropoietin levels and increased renewal rate of erythrocytes occur during pregnancy (Lurie and Mamet 2000) and particularly in severe preeclampsia (Troeger, Holzgreve et al. 2006).

The results of this study seem quite consistent, especially because they also included increases in the blood levels of creatinine, uric acid and ferritin in the group of pregnant women with severe preeclampsia, as commonly occurs in this pathologic condition. (Barton and Sibai 2004, Berhan 2016).

In summary, although the populations of these study groups are small, we can say that both in the gestational hypertension and in severe pre-eclampsia there is preservation of the erythrocyte membrane stability, although by different mechanisms. These study findings suggest that the maintenance of the erythrocytes stability in gestational hypertension is associated with lipid exchange with lipoproteins, while the stability increase observed in severe preeclampsia is associated with increase in the erythrocytes turnover.

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References

1 Carvalho MA, Faundes A and Santos LC. Pregnancy-induced hypertension and hyaline membrane disease. *Int J Gynaecol Obstet* 1997;**58**:197-202. https://doi.org/10.1016/S0020-7292(97)00066-0

2 Leeman L and Fontaine P. Hypertensive Disorders of Pregnancy. *American Family Physician* 2008;**78**:93-100.

3 Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol* 2013;**122**:1122-31.

4 Magee LA, Pels A, Helewa M, Rey E, von Dadelszen P and Committee SHG. Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy: executive summary. *J Obstet Gynaecol Can* 2014;**36**:575-6. https://doi.org/10.1016/S1701-2163(15)30533-8

5 Canessa M, Adragna N, Solomon HS, Connolly TM and Tosteson DC. Increased sodium-lithium countertransport in red cells of patients with essential hypertension. *N Engl J Med* 1980;**302**:772-6. https://doi.org/10.1056/NEJM198004033021403

6 Tsuda K, Minatogawa Y, Tsuda S, Shima H, Nishio I, Kido R *et al.* Alterations in calcium-handling of erythrocytes in spontaneously hypertensive rats. Calcium-induced changes in the osmotic fragility of erythrocytes in hypertension. *Jpn Heart J* 1987;**28**:923-31. https://doi.org/10.1536/ihj.28.923

7 Zicha J, Kunes J and Devynck MA. Abnormalities of membrane function and lipid metabolism in hypertension: a review. *Am J Hypertens* 1999;**12**:315-31. https://doi.org/10.1016/S0895-7061(98)00178-2

8 Mohandas N and Evans E. Mechanical properties of the red cell membrane in relation to molecular structure and genetic defects. *Annu Rev Biophys Biomol Struct* 1994;**23**:787-818. https://doi.org/10.1146/annurev.bb.23.060194.004035

9 Mohandas N and Evans E. Mechanical Properties of the Red Cell Membrane in Relation to Molecular Structure and Genetic Defects. *Annual Review of Biophysics and Biomolecular Structure* 1994;**23**:787-818. https://doi.org/10.1146/annurev.bb.23.060194.004035

10 Penha-Silva N, Arvelos LR, Cunha CC, Aversi-Ferreira TA, Gouvea-e-Silva LF, Garrote-Filho MS *et al.* Effects of glycerol and sorbitol on the thermal dependence

of the lysis of human erythrocytes by ethanol. *Bioelectrochemistry* 2008;**73**:23-9. https://doi.org/10.1016/j.bioelechem.2008.04.002

11 Penha-Silva N, Firmino CB, de Freitas Reis FG, da Costa Huss JC, de Souza TM, de Freitas MV *et al.* Influence of age on the stability of human erythrocyte membranes. *Mech Ageing Dev* 2007;**128**:444-9. https://doi.org/10.1016/j.mad.2007.06.007

12 Cunha CC, Arvelos LR, Costa JO and Penha-Silva N. Effects of glycerol on the thermal dependence of the stability of human erythrocytes. *J Bioenerg Biomembr* 2007;**39**:341-7. https://doi.org/10.1007/s10863-007-9092-z

13 Pacetti D, Gagliardi R, Balzano M, Frega NG, Ojeda ML, Borrero M *et al.* Changes in the Fatty Acid Profile and Phospholipid Molecular Species Composition of Human Erythrocyte Membranes after Hybrid Palm and Extra Virgin Olive Oil Supplementation. *J Agric Food Chem* 2016. https://doi.org/10.1021/acs.jafc.6b01903

14 Cooper RA, Leslie MH, Fischkoff S, Shinitzky M and Shattil SJ. Factors influencing the lipid composition and fluidity of red cell membranes in vitro: production of red cells possessing more than two cholesterols per phospholipid. *Biochemistry* 1978;**17**:327-31. https://doi.org/10.1021/bi00595a021

15 Fessler MB, Rose K, Zhang Y, Jaramillo R and Zeldin DC. Relationship between serum cholesterol and indices of erythrocytes and platelets in the US population. *J Lipid Res* 2013;**54**:3177-88. https://doi.org/10.1194/jlr.P037614

16 Cooper RA. Anemia with Spur Cells - a Red Cell Defect Acquired in Serum and Modified in Circulation. *Journal of Clinical Investigation* 1969;**48**:1820-&. https://doi.org/10.1172/JCI106148

17 Bernardino Neto M, de Avelar EB, Jr., Arantes TS, Jordao IA, da Costa Huss JC, de Souza TM *et al.* Bivariate and multivariate analyses of the correlations between stability of the erythrocyte membrane, serum lipids and hematological variables. *Biorheology* 2013;**50**:305-20. https://doi.org/10.3233/BIR-130641

18 de Freitas MV, Marquez-Bernardes LF, de Arvelos LR, Paraiso LF, Goncalves EOAF, Mascarenhas Netto Rde C *et al.* Influence of age on the correlations of hematological and biochemical variables with the stability of erythrocyte membrane in relation to sodium dodecyl sulfate. *Hematology* 2014;**19**:424-30. https://doi.org/10.1179/1607845413Y.0000000145

19 de Arvelos LR, Rocha VC, Felix GP, da Cunha CC, Bernardino Neto M, da Silva

Garrote Filho M *et al.* Bivariate and multivariate analyses of the influence of blood variables of patients submitted to Roux-en-Y gastric bypass on the stability of erythrocyte membrane against the chaotropic action of ethanol. *J Membr Biol* 2013;**246**:231-42. https://doi.org/10.1007/s00232-013-9524-0

20 Tziakas D, Chalikias G, Grapsa A, Gioka T, Tentes I and Konstantinides S. Red blood cell distribution width: a strong prognostic marker in cardiovascular disease: is associated with cholesterol content of erythrocyte membrane. *Clin Hemorheol Microcirc* 2012;**51**:243-54. https://doi.org/10.3233/CH-2012-1530

21 Netto RDM, Fabbri C, de Freitas MV, Neto MB, Garrote MS, Lacerda MVG *et al.* Influence of Plasmodium vivax malaria on the relations between the osmotic stability of human erythrocyte membrane and hematological and biochemical variables. *Parasitology Research* 2014;**113**:863-74. https://doi.org/10.1007/s00436-013-3717-4

22 de Freitas MV, Marquez-Bernardes LF, de Arvelos LR, Paraiso LF, Goncalves E, F. OA *et al.* Influence of age on the correlations of hematological and biochemical variables with the stability of erythrocyte membrane in relation to sodium dodecyl sulfate. *Hematology* 2014;**19**:424-30. https://doi.org/10.1179/1607845413Y.0000000145

23 Patel KV, Ferrucci L, Ershler WB, Longo DL and Guralnik JM. Red blood cell distribution width and the risk of death in middle-aged and older adults. *Arch Intern Med* 2009;**169**:515-23. https://doi.org/10.1001/archinternmed.2009.11

24 Patel KV, Mohanty JG, Kanapuru B, Hesdorffer C, Ershler WB and Rifkind JM. Association of the red cell distribution width with red blood cell deformability. *Adv Exp Med Biol* 2013;**765**:211-6. https://doi.org/10.1007/978-1-4614-4989-8_29

25 Patel KV, Semba RD, Ferrucci L, Newman AB, Fried LP, Wallace RB *et al.* Red cell distribution width and mortality in older adults: a meta-analysis. *J Gerontol A Biol Sci Med Sci* 2010;**65**:258-65. https://doi.org/10.1093/gerona/glp163

26 Malandrino N, Wu WC, Taveira TH, Whitlatch HB and Smith RJ. Association between red blood cell distribution width and macrovascular and microvascular complications in diabetes. *Diabetologia* 2012;**55**:226-35. https://doi.org/10.1007/s00125-011-2331-1

27 Zalawadiya SK, Veeranna V, Panaich SS and Afonso L. Red cell distribution width and risk of peripheral artery disease: analysis of National Health and

Nutrition Examination Survey 1999-2004. *Vasc Med* 2012;**17**:155-63. https://doi.org/10.1177/1358863X12442443

28 Tziakas DN, Chalikias GK, Stakos D and Boudoulas H. The role of red blood cells in the progression and instability of atherosclerotic plaque. *Int J Cardiol* 2010;**142**:2-7. https://doi.org/10.1016/j.ijcard.2009.10.031

29 Zalawadiya SK, Zmily H, Farah J, Daifallah S, Ali O and Ghali JK. Red cell distribution width and mortality in predominantly African-American population with decompensated heart failure. *J Card Fail* 2011;**17**:292-8. https://doi.org/10.1016/j.cardfail.2010.11.006

30 Paraiso LF, de Freitas MV, Goncalves EOAF, de Almeida Neto OP, Pereira EA, Mascarenhas Netto RC *et al.* Influence of acute exercise on the osmotic stability of the human erythrocyte membrane. *Int J Sports Med* 2014;**35**:1072-7. https://doi.org/10.1055/s-0034-1371834

31 Hernandez Hernandez JD, Villasenor OR, Del Rio Alvarado J, Lucach RO, Zarate A, Saucedo R *et al.* Morphological changes of red blood cells in peripheral blood smear of patients with pregnancy-related hypertensive disorders. *Arch Med Res* 2015;**46**:479-83. https://doi.org/10.1016/j.arcmed.2015.07.003

32 Hofmeyr GJ and Belfort M. Proteinuria as a predictor of complications of pre-eclampsia. *BMC Med* 2009;**7**:11. https://doi.org/10.1186/1741-7015-7-11

33 Lurie S and Mamet Y. Red blood cell survival and kinetics during pregnancy. *Eur J Obstet Gynecol Reprod Biol* 2000;**93**:185-92. https://doi.org/10.1016/S0301-2115(00)00290-6

34 Troeger C, Holzgreve W, Ladewig A, Zhong XY and Hahn S. Examination of maternal plasma erythropoietin and activin A concentrations with regard to circulatory erythroblast levels in normal and preeclamptic pregnancies. *Fetal Diagn Ther* 2006;**21**:156-60. https://doi.org/10.1159/000089068

35 Chabanel A, Flamm M, Sung KL, Lee MM, Schachter D and Chien S. Influence of cholesterol content on red cell membrane viscoelasticity and fluidity. *Biophys J* 1983;**44**:171-6. https://doi.org/10.1016/S0006-3495(83)84288-X

36 Bagdade JD, Buchanan WF, Pollare T and Lithell H. Abnormal lipoprotein phospholipid composition in patients with essential hypertension. *Atherosclerosis* 1995;**117**:209-15. https://doi.org/10.1016/0021-9150(95)05573-F

37 Vazquez-Rodriguez JG, Rios-Gutierrez CD, Paredes-Lozano EP and Garcia-Flores A. [Frequency and maternal complications of the criteria of hemolysis in

preeclamptic patients with HELLP syndrome treated in an intensive care unit]. *Ginecol Obstet Mex* 2016;**84**:19-26.

38 Catanzarite VA, Steinberg SM, Mosley CA, Landers CF, Cousins LM and Schneider JM. Severe preeclampsia with fulminant and extreme elevation of aspartate aminotransferase and lactate dehydrogenase levels: high risk for maternal death. *Am J Perinatol* 1995;**12**:310-3. https://doi.org/10.1055/s-2007-994482

39 Barton JR and Sibai BM. Diagnosis and management of hemolysis, elevated liver enzymes, and low platelets syndrome. *Clin Perinatol* 2004;**31**:807-33, vii. https://doi.org/10.1016/j.clp.2004.06.008

40 Berhan Y. No Hypertensive Disorder of Pregnancy; No Preeclampsia-eclampsia; No Gestational Hypertension; No Hellp Syndrome. Vascular Disorder of Pregnancy Speaks for All. *Ethiop J Health Sci* 2016;**26**:177-86. https://doi.org/10.4314/ejhs.v26i2.12

Table 1 Baseline characteristics of the study population

Variables	Control (N=9)	Gestacional Hypertension (N=7)	Superimposed Preeclampsia (N=7)	Severe Preeclampsia (N=9)
Maternal age (years)	20 (6)	31 (7)	32 (18)	33 (7)
Gestacional age (weeks)	40.7 (0.75)	37 (4.5)	38 (15)	33.7 (9.5)
SBP (mmHg)	115 (10)	150 (00)	145 (40)	160 (10)
DBP (mmHg)	73 (10)	90 (10)	100 (10)	110 (20)
Ht (%)	34.5 (3.8)	38.4 (8.5)	35.1 (6.7)	36.9 (5.4)
Hb (mg/dL)	11.3 (1.7)	12.6 (3.0)	11.8 (2.7)	12.3 (2.0)
RBC (10^6/mm^3)	4.33 (0.45)	4.21 (0.7)	4.16 (1.2)	4.15 (0.9)
MCV (fL)	80.8 (9.2)	89.1 (11.3)	87.0 (7.5)	87.3 (5.0)
RDW (%)	14.0 (1.0)	13.0 (1.0)	13.0 (1.0)	13.0 (3.0)
MCH (pg)	27.0 (4.4)	29.9 (3.2)	30.6 (3.1)	28.8 (1.0)
MCHC (%)	33.4 (1.3)	33.6 (1.6)	33.8 (1.7)	33.5 (0.8)
RI (%)	1.8 (2.1)	0.85 (0.3)	1.0 (1.8)	0.65 (0.8)
Plt (10^3/mm^3)	2.65 (8.6)	1.79 (6.6)	1.66 (7.8)	2.09 (7.3)
MPV (fL)	7 (2)	9 (3)	9 (1)	8 (2)
PT (%)	89.85 (20.3)	1 (49.56)	0.99 (0.03)	1 (54)
t-C (mg/dL)	237 (69)	271.5 (73)	214 (93)	211 (35)
TGC (mg/dL)	191.5 (72)	221 (122)	204 (128)	166 (146)
VLDL-C (mg/dL)	39 (4)[a,d]	67.4 (33.82)[b,d]	48 (36.6)	30 (11.8)[a,b]
LDL-C (mg/dL)	137 (58)	102.6 (62.3)	112.9 (47)	131.15 (38.25)
HDL-C (mg/dL)	67 (14)	69.45 (24.9)	66.5 (20.8)	61.5 (8.5)
AST (IU/L)	14.4 (2.6)[d]	12 (5)[a]	15 (4)	18 (6)[a,d]
ALT (IU/L)	7 (2) [a,b]	8 (6)[d]	11 (4)[a]	13 (6)[b,d]
LDH (IU/L)	215 (50)[d,e]	176.5 (35)[a,d]	183 (35)[b,e]	255 (73)[a,b]
AF (IU/L)	-	125 (62)	108 (68)	76.5 (50)
Urea (mg/dL)	15.5 (3.6)	22 (14)	17 (25)	27 (26.5)
Creatinine (mg/dL)	0.49 (0.09)[a,b,c]	0.7 (0.2)[a]	0.6 (0.1)[b,d]	0.7 (0.1)[c,d]
Uric Acid (mg/dL)	4.6 (0.4)[a]	5.09 (1.52)[d]	4.55 (2.52)[b]	6.65 (1.49)[a,b,d]
Na^+ (mEq/L)	138 (2)	137 (4)	137 (5)	139 (2)
K^+ (mEq/L)	4.25 (0.57)	4.1 (1)	4 (1.2)	4.1 (0.7)
iB (mg/dL)	0.20 (0.08)	0.15 (0.035)	0.17 (0.08)	0.28 (0.27)
tB (mg/dL)	0.3 (0.01)	0.28 (0.08)	0.31 (0.3)	0.39 (0.34)
Fe (µg/dL)	65 (45)	119.1 (93.2)	73.7 (5.8)	82.1 (57.05)
Ferritin (ng/mL)	17.55 (16.79)[a,d]	67.43 (0)	60.44 (41.53)[d]	84.25 (185.48)[a]
HSA (g/dL)	3.495 (0.26)[a]	3 (0)	-	3.07 (0.52)[a]

[a,b and c] and [d and e] indicate statistically significant ($p<0.05$) and borderline differences ($0.05<p<0.10$), respectively, when present as pairs of common letters.

Abbreviations: SPB, systolic blood pressure; DBP, diastolic blood pressure; A_{max}, absorbance at 540 nm associated with lysis of the whole population of erythrocytes; A_{min}, absorbance at 540 nm associated with residual lysis of the erythrocytes population; $1/H_{50}$, inverse the NaCl concentration capable of promoting 50% haemolysis; dX, variation in the concentration of NaCl responsible for total haemolysis; N, number of participants; Ht, hematocrit; Hb, hemoglobin; RBC, erythrocytes; MCV, mean corpuscular volume; RDW, red cell distribution width; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RI, reticulocytes index; Plt, platelets; MPV, mean platelet volume; PT, prothrombin time; t-C, total cholesterol; TGC, triglycerides; VLDL-C, very low density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; AF, alkaline fosfatase; Na^+, sodium; K^+, potassium; iB, indirect bilirubin; tB, total bilirubin; Fe, serum iron; HSA, human serum albumin.

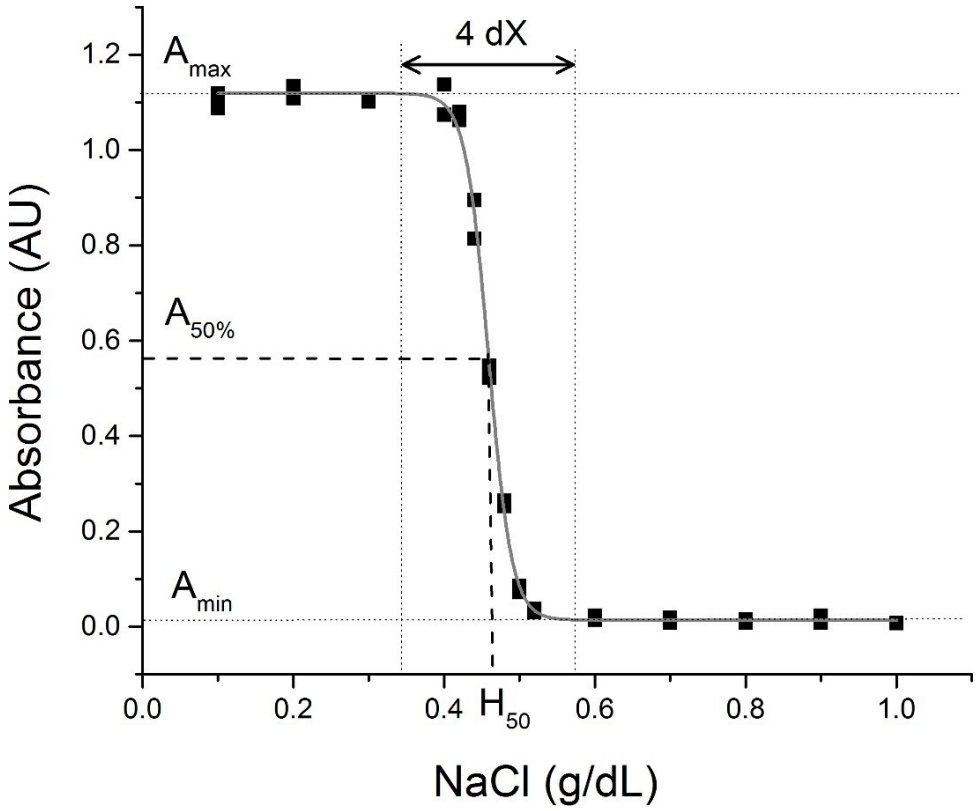

Figure 1: Typical curve of osmotic fragility haemolysis. A_{min} and A_{max} represent respectively the minimum and maximum mean value of absorbance. H_{50} is the NaCl concentration capable of promoting 50% haemolysis. dX is the NaCl concentration range responsible for 100% haemolysis.

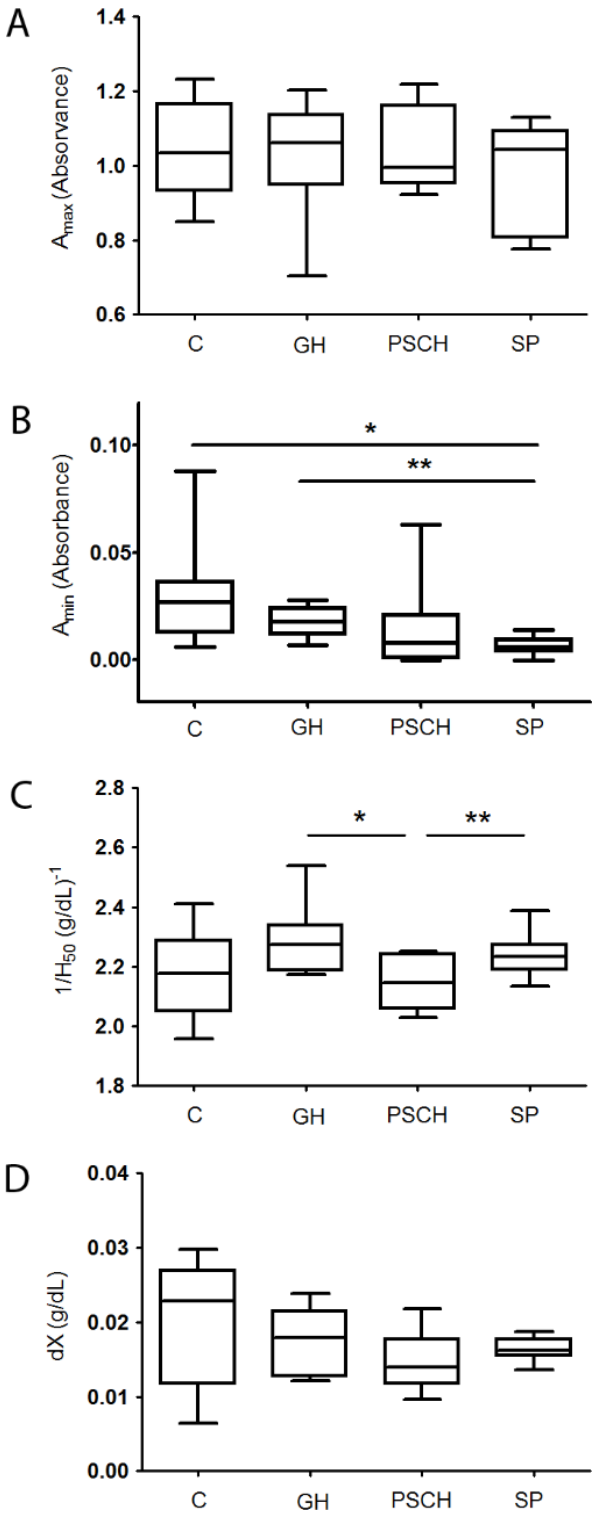

Figure 2: Comparison of the osmotic stability parameters A_{min} (**A**), A_{max} (**B**), $1/H_{50}$ (**C**) and dX (**D**) between groups (C, control; GH, gestational hypertension; PSCH, preeclampsia superimposed on chronic hypertension; SP, severe preeclampsia). *$p<0.05$ indicates statistically significant difference and **$0.05<p<0.10$ indicates borderline difference.