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TRABALHO FINAL COM VISTA À ATRIBUIÇÃO DO GRAU DE MESTRE NO ÂMBITO DO CICLO DE ESTUDOS DE MESTRADO INTEGRADO EM MEDICINA

GILBERTO JOÃO PADILHA MARQUES

RECEPTOR SOLÚVEL DA TRANSFERRINA: UTILIDADE CLÍNICA NO DIAGNÓSTICO DE ANEMIA SIDEROPÉNICA E SUA APLICAÇÃO NA ROTINA LABORATORIAL

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LISTA DE ABREVIATURAS

ADC – Anemia das doenças crónicas

AMO – Aspirado de medula óssea

AR – Artrite reumatóide

AS – Anemia sideropénica

CHr – Conteúdo de hemoglobina reticulocitária

DFF – Deficiência funcional de ferro

EIA – Imunoensaio enzimático

ELISA – *Enzime linked immunosorbent assay*

Hb – Hemoglobina

HCM – Hemoglobina corpuscular média

HIV – Vírus da imunodeficiência humana

Índice sTfR-F – índice sTfR/logFerritina

MO – Medula óssea

PCR – Proteína C reactiva

sTfR – Receptor solúvel da transferrina

TfR – Receptor da transferrina

VCM – Volume corpuscular médio

VPN – Valor preditivo negativo

VPP – Valor preditivo positivo

VS – Velocidade de sedimentação eritrocitária

RESUMO

Introdução: O diagnóstico diferencial de anemia sideropénica (AS) e anemia das doenças crónicas (ADC) é, por vezes, difícil. O estudo do aspirado da medula óssea é o padrão na avaliação das reservas de ferro. No entanto, devido ao consumo de tempo e recursos, é impraticável na rotina diária. Na rotina laboratorial são utilizados marcadores séricos como a ferrémia, a saturação da transferrina ou a ferritina, sendo esta o principal marcador sérico das reservas de ferro. Contudo, como proteína de fase aguda, os seus níveis aumentam na presença de inflamação/infecção, dificultando uma distinção clara entre AS e ADC. Os outros marcadores séricos "clássicos" tem, actualmente, pouco valor diagnóstico.

A quantificação do receptor solúvel da transferrina (sTfR) é considerada útil na avaliação de sideropenia. Muitos defendem que o uso simultâneo do sTfR e da ferritina (índice sTfR/logFerritina – sTfR-F) é o melhor indicador de sideropenia, principalmente na presença de inflamação ou infecção. Não há, contudo, consenso relativamente ao seu uso na investigação de rotina de anemias.

Conteúdo: Após pesquisa na "PubMed" foram revistos 19 artigos relativos ao uso do sTfR e/ou do índice sTfR-F no diagnóstico diferencial entre AS e ADC com o objectivo de avaliação do uso destes no diagnóstico de sideropenia na rotina laboratorial.

Sumário: O uso do sTfR e do do índice sTfR-F no diagnóstico de AS em doentes em fase aguda, com ferritinémia normal ou alta, permite o diagnóstico de sideropenias que, de outra forma, seriam subdiagnosticadas. É, no entanto, necessária uma padronização dos ensaios do sTfR para o seu uso generalizado.

Palavras-chave: Receptor solúvel da transferrina; Anemia sideropénica; Ferritina; Doença Crónica; Aspirado de medula óssea

ABSTRACT

Background: The differential diagnosis between iron deficiency anemia (IDA) and anemia of chronic disease (ACD) is, sometimes, difficult. Examination of bone marrow aspirates (BMA) is the "gold standard" in the evaluation of body iron stores, although, being time and resource consuming, it is unsuitable for routine use. Hence surrogate serum markers, like serum iron, transferring saturation and ferritin, are used. The latter is considered the best serum marker of body iron stores, but, as an acute-phase protein, its interpretation in the presence of inflammation is not possible and it may not distinguish IDA from ACD. The other "classic" serum markers had no additional diagnostic value to serum ferritin (SF), nowadays.

The serum levels of soluble transferring receptor (sTfR) are considered useful in the investigation of iron deficiency. Many authors agree that the simultaneous use of sTfR and ferritin (sTfR/logSF index – sTfR-F Index) is the best indicator of IDA in the presence of inflammation or infection. However, there is no consensus over its use in the routine investigation of anemia.

Content: Following a PubMed search, we reviewed 19 articles on the use of sTfR and sTfR-F Index in the differential diagnosis of IDA and ACD with the objective of evaluating its use in the routine investigation of iron deficiencies.

Summary: The use of sTfR and sTfR-F Index in the diagnosis of IDA during acute phase reactions, with normal to high SF levels, might allow detection of otherwise undiagnosed iron deficiencies. Nevertheless, the lack of standardization on sTfR assays must be address.

Keywords: Receptors, Transferrin; Anemia, Iron-Deficiency; Ferritins; Chronic Disease; Bone Marrow

INTRODUÇÃO

A anemia sideropénica (AS) é a forma mais frequente de anemia, seguida da anemia das doenças crónicas (ADC) (1). Uma vez que um diagnóstico de sideropenia pode envolver investigações complementares (2,3), por vezes invasivas (como as técnicas endoscópicas) e implicando o consumo de tempo e recursos, o seu diagnóstico deve ser cuidadoso (4). Além disso, uma terapia inapropriada com ferro em doentes com ADC pode agravar a doença subjacente (5). Contudo, o diagnóstico correcto destas anemias é, por vezes, difícil (6). As duas situações podem apresentar indíces eritrocitários semelhantes, tais como um volume corpuscular médio (VCM) com microcitose, e uma hemoglobina corpuscular média (HCM) com hipocromia (7). A realização de aspirado de medula óssea (AMO), corado com Azul da Prússia (coloração de Perls) para detecção de depósitos de hemossiderina, é, ainda hoje, considerado o padrão para a avaliação das reservas corporais de ferro (2,5,8,9) – sendo a ausência de ferro na medula óssea (MO) considerada como um indicador de sideropenia (4). Mas, sendo invasiva, cara, trabalhosa (7) e dependente do operador (10,11), a sua aplicação na investigação laboratorial de rotina de anemias torna-se impraticável (9). Assim, recorre-se a marcadores séricos para avaliar as reservas de ferro (5), tais como a ferritina, a ferrémia, a capacidade total de fixação do ferro e a saturação da transferrina. Não há nenhum marcador único, cujo uso isolado permita esta avaliação (4,12). A ferritinémia é considerada o melhor marcador sérico para a estimativa das reservas férricas (9,13–15) tendo sido estabelecida como um indicador preciso dos níveis corporais de ferro (7) em indivíduos sem comorbilidades. Perante uma sideropenia, na ausência de patologia inflamatória/infecciosa coexistente, a ferritinémia apresenta-se abaixo dos 12-15 μg/L (16). No entanto, a ferritina é uma proteína de fase aguda (2,14), cujos níveis séricos aumentam em situações de inflamação ou infecção, perdendo-se assim a sua utilidade

na avaliação das reservas de ferro (16). A interpretação da ferritinémia em fase aguda pode ser difícil (4,14). O doseamento da ferritina sérica deve ser acompanhado por doseamentos de marcadores de fase aguda, como a proteína C reactiva (PCR) e/ou a velocidade de sedimentação eritrocitária (VS), para exclusão de níveis elevados devido a situações inflamatórias ou infecciosas (4,17).

Outros marcadores séricos "clássicos", como a ferrémia, a capacidade total de fixação do ferro ou a saturação da transferrina oferecem pouca, ou nenhuma, informação adicional relativamente à ferritina (14) tendo, igualmente, respostas de fase aguda, pelo que o seu uso na investigação de anemias é limitado (2). Para além disso, a ferrémia flutua com o ritmo circadiano e a alimentação (18), limitando ainda mais o seu uso na investigação de sideropenia.

Apesar do fácil diagnóstico diferencial entre as formas "puras" de AS e ADC com os marcadores séricos clássicos (19), a possibilidade de coexistência destas duas entidades (7,19) pode tornar pouco clara a sua distinção se limitados ao uso destes marcadores (4).

O receptor solúvel da transferrina (sTfR) é uma forma truncada do receptor tecidular da transferrina, a proteína transportadora de ferro (20). O receptor da transferrina (TfR) é uma glicoproteína transmembranar, de 760 aminoácidos. A sua forma funcional é composta por dois monómeros ligados por duas pontes dissulfito para formar uma molécula de 190,000 Da (15). Num adulto normal, cerca de 80% do TfR está presente nos precursores eritróides da MO. A massa total de TfR celular depende, portanto, do número de precursores eritróides na MO e do número de TfR por célula (variável em função das necessidades celulares de ferro) (15).

A concentração sérica do sTfR é proporcional à expressão membranar do TfR (21) sendo induzida pela privação de ferro às células (4,13). Os eritroblastos e, em menor grau, os reticulócitos são a principal origem do sTfR circulante (15,22). Não sendo uma proteína de fase aguda (3,4,23), os níveis séricos de sTfR não são afectados pela presença de inflamação ou infecção. Foi demonstrado que não há indução da síntese de TfR nas células eritrocitárias na ADC (24). Ao contrário da ferritina, os valores do sTfR, nos adultos, não variam nem com o sexo nem com a idade (14,15,25).

O nível sérico do sTfR apresenta uma forte correlação com a depleção das reservas medulares de ferro em indivíduos sem comorbilidades (13,26), no entanto, os seus aumentos não se limitam a situações de sideropenia (4) ocorrendo, igualmente, em distúrbios associados a estimulação da eritropoiese – como talassémias (14,27), anemias hemolíticas (2) e anemias falciformes (28) –, na eritropoiese ineficaz - anemias megaloblásticas (2) e síndromes mielodisplásicos (26) –, nas leucemias crónicas (16) e agudas (4), e na terapêutica com eritropoietina. A diminuição dos níveis de sTfR associa-se aos síndromes de falência medular (15), ao défice de eritropoietina – como na insuficiência renal crónica (29) – e ao excesso de ferro (15). Estas patologias devem ser consideradas na interpretação dos resultados.

Em contraste com a ferritina, que reflecte os compartimentos de reservas de ferro (30), o sTfR avalia o suprimento medular de ferro (2) e o grau de eritropoiese (14), reflectindo o compartimento funcional de ferro (30,31). O uso de razões matemáticas baseadas nestes dois parâmetros – como o índice sTfR/logFerritina (índice sTfR-F) e a razão sTfR/Ferritina – são apontadas por alguns autores como uma boa ferramenta para a estimativa das reservas de ferro, conjugando dois fenómenos que ocorrem durante o esgotamento das mesmas: a diminuição da ferritina e o aumento do sTfR (2,23,30).

Deste modo, estes autores defendem o seu uso em casos de inflamação/infecção e anemia concomitantes.

Kohgo et al., em 1987, descreveram o primeiro imunoensaio para doseamento dos níveis séricos de sTfR (32), e várias metodologias alternativas foram desenvolvidas desde então. Enquanto as primeiras metodologias – incluindo radioimunoensaio e ELISA (33,34) – eram muito trabalhosas e dependentes da intervenção do operador (35), o desenvolvimento de ensaios imunoturbidimétricos (35) e imunonefelométricos (33), passíveis de automatização (com uma boa precisão), tornou possível o doseamento do sTfR em laboratórios clínicos (34,35), em pequeno volume de soro (34), com resultados em pouco tempo (36).

Contudo, existe grande variabilidade entre os valores obtidos com os ensaios dos diversos fabricantes, variabilidade esta atribuível ao uso de reagentes com anticorpos mono- *vs* policlonais (8) e ao uso de calibradores constituídos por moléculas de TfR intactas *vs* na sua forma complexada com transferrina (16,33).

MÉTODOS

Foi realizada uma pesquisa no motor de busca da "PubMed" utilizando os termos Booleanos: ["soluble transferrin receptor" AND "iron deficiency anemia"] e uma segunda pesquisa Booleana pelos termos MeSH (*Medical Subject Headings*): ["Receptors, Transferrin" AND "Anemia, Iron-Deficiency"].

Dos artigos encontrados foram seleccionados aqueles em que foi usado o estudo do AMO como padrão de avaliação das reservas de ferro, ou em que houve uma

classificação, bem definida, dos doentes estudados nos grupos com AS, ADC ou ADC com AS, com base em critérios clínicos e laboratoriais. Os artigos em que a definição destes grupos foi baseada apenas em marcadores bioquímicos "clássicos" (ferritinémia, ferro sérico, saturação de transferrina e marcadores de fase aguda), foram excluídos uma vez que, como mostrado anteriormente, é muitas vezes impossível o diagnóstico diferencial entre a AS e a ADC recorrendo unicamente a esses marcadores (4), resultando em viés que podem influenciar os resultados dos estudos em causa. Preencheram estes critérios de inclusão 19 artigos (Tabela 1).

REVISÃO DA LITERATURA E DISCUSSÃO

Em 1997, Punnonen et al. (2) publicaram um artigo em que estudaram 129 doentes com anemia (Tabela 1). Foram definidos grupos com AS (n=48), com ADC (n=64) e com AS+ADC (n=17), baseados em AMO para a classificação das anemias. O sTfR foi doseado por imunoensaio enzimático (EIA) policlonal. O índice sTfR-F (com um *cut-off* de 1,5) teve uma sensibilidade de 98% e uma especificidade de 100%, ambas superiores ao sTfR com *cut-off* de (2,7 mg/L) (94% nos dois parâmetros) e à ferritina com *cut-off* de 41 μg/L (91% e 98%, respectivamente), revelando-se um excelente marcador de sideropenia.

No ano seguinte, Mast et al. (14), obtiveram resultados semelhantes estudando uma amostra não seleccionada de 54 doentes anémicos submetidos a AMO, identificando uma sensibilidade e especificidade do sTfR (*cut-off* de 2,8 mg/L; doseado por EIA policlonal) no diagnóstico de sideropenia foi de 100% e de 86%, respectivamente, com um valor preditivo positivo (VPP) de 42% e negativo (VPN) de 100%.

Artigo	Amostra de estudo (número de doentes)	Critérios de exclusão
Punnonen (1997)	Doentes submetidos a AMO para estudo de anemia (n=129)	Terapêutica com ferro; doença hemato-oncológica; anemia hemolítica; deficiência em vitamina B12 ou folato
Junca (1998)	Doentes com anemia e patologia infecciosa ou inflamatória (n=37)	Sem critérios de exclusão
Mast (1999)	Doentes com anemia submetidos a AMO (n=54)	Sem critérios de exclusão
Means (1999)	Doentes submetidos a AMO (n=145)	Terapêutica com ferro ou eritropoietina; hemólise; deficiência em vitamina B12
Suominen (2000)	Doentes com anemia e AR (n=30)	Sem critérios de exlcusão
Ruivard (2000)	Doentes internados (com ou sem anemia) (n=54)	Invasão medular por mais de 30% de células anómalas; menos que 5 fragmentos medulares
Joosten (2002)	Doentes idosos hospitalizados (n=83)	Doença hemato-oncológica; hemólise; deficiência em vitamina B12 ou folato; insuficiência renal; terapêutica com ferro; transfusão ou cirurgia cirurgia recentes
Rimon (2002)	Doentes com internados, com mais de 80 (n=63)	Ausência de consentimento; terapêutica com ferro; deficiência em vitamina B12 ou folato; HDA; neoplasias; insuficiência renal ou hepática
Fitzsimons (2002)	Doentes com anemia e AR comparado com IDA e indivíduos saudáveis (n=44)	Sem critérios de exclusão
Lee (2002)	Doentes com anemia e neoplasia não-hematológica ou inflamação crónica concomitantes (n=120)	Doença hemato-oncológica; hemólise; deficiência em vitamina B12 ou folato; terapêutica com ferro; hipercelularidade medular
Baillie (2003)	Doentes com artrite reumatóide e doentes com anemia (n=120)	Sem descrição de critérios de exclusão
Hanif (2005)	Doentes adultos com anemia submetidos a AMO (n=176)	Talassémia; anemia sideroblástica
Chang (2007)	Doentes submetidos a AMO (n=76)	Insuficiência renal; SMD; anemia hemolítica; policitémia; terapêutica com eritropoietina; transfusões recentes;
Phiri (2009)	Crianças com anemia grave (n=381)	Sem critérios de exclusão
Karlsson (2010)	Doentes com mais de 60 anos e anemia (n=50)	Suplementação com ferro; transfusões recentes
Jain (2010)	Crianças abaixo dos 18 anos (n=60)	Doença hemato-oncológica; anemia hemolítica; insuficiência renal; patologia hepática ou endócrina; deficiência em vitamina B12 ou folato; hemorragia aguda, transfusão recente ou suplementação com ferro.
Berlin (2011)	Doentes com anemia em hospitalização aguda e sTfR aumentado com ferritina normal ou alta (n=32)	Sem critérios de exclusão
Karlsson (2011)	Doentes idosos com anemia (n=54)	Suplementação com ferro; transfusões recentes; leucemias; mieloma múltiplo; SMD; deficiência em vitamina B12 ou folato; anemia hemolítica
Skikne (2011)	Doentes com anemia (n=145)	Anemia hemolítica; deficiência B12/folato; hemorragia aguda recente; doença hemato-oncológica; infecção HIV; traumatismo; quimioterapia, hemodiálise; terapêutica com ferro, eritropoietina ou micofenolato mofetil

Tabela 1. Caracterização das populações estudadas, com descrição dos critérios de exclusão usados pelos diferentes autores. AR: Artrite reumatóide; SMD: síndrome mielodisplásico; AMO: aspirado de medula óssea

A ferritina, com *cut-off* de 12 μg/L, teve valores de sensibilidade e especificidade de 20% e 98%, respectivamente, com VPP de 50% e VPN de 92%; o aumento do *cut-off* para 30 μg/L melhorou a sensibilidade para 100%, mantendo a mesma especificidade. Os autores concluíram que o sTfR não era superior à ferritina com um *cut-off* de 30 μg/L na investigação de rotina de doentes com suspeita de sideropenia, reservando o seu uso para patologias de fase aguda concomitantes.

Em contraste com as conclusões de Punnonen e de Mast, Junca et al. (19), também em 1998, numa amostra de 10 doentes hipoferritinémicos (<25 μg/L), 12 doentes hiperferritinémicos com alterações inflamatórias/infecciosas e poucas ou nenhumas reservas de ferro medular (por AMO), e 15 doentes hiperferritinémicos com alterações inflamatórias/infecciosas e valores normais ou aumentados de ferro medular, obtiveram uma sensibilidade e especificidade para o sTfR (*cut-off* 4,5 mg/L) de apenas 50% e 74%, respectivamente. Observaram que o sTfR (por EIA) detectou sideropenia, no contexto de ADC, em apenas metade dos casos, e que 4 dos 15 casos com ferro medular normal apresentavam níveis séricos de sTfR acima do valor de referência (nenhum deles com hiperplasia eritróide). Contudo, este estudo baseou-se numa pequena amostra e incluiu no mesmo grupo doentes com reservas de ferro medular reduzidas e ausentes, aspecto que pode ser uma fonte de viés; estas características do estudo podem explicar a diferença de resultados face a outras publicações.

Em 1999, Means et al. (11) publicaram os resultados de um estudo tricêntrico com 145 doentes submetidos a AMO (a maior parte deles para estadiamento de doença oncológica e apenas 7% para diagnóstico de anemia), dos quais apenas 24 não apresentavam reservas de ferro medular à coloração de Perls. Os autores verificaram que o sTfR (doseado por ELISA) foi o único teste em que os doentes sideropénicos

tinham valores médios fora do intervalo de referência, e os não-sideropénicos dentro do mesmo intervalo. Contudo, apesar da maior sensibilidade do sTfR (com um *cut-off* de 28,1 e 30 nmol/L – 2,07 e 2,2 mg/L – para não-afro-americanos e afro-americanos, respectivamente) face à ferritina (25%, com um *cut-off* de 10 e 22 μg/L para mulheres e homens, respectivamente), esta era de apenas 71%, com uma especificidade de 74%, inferior à da ferritina (99%). Os autores apresentaram um algoritmo para a previsão das reservas de ferro medular baseado no doseamento sequencial da ferritinémia e do sTfR. Assumiram, baseados nos resultados de North et al. (37), que um nível de ferritinémia <25 μg/L indicava ausência de ferro medular e que uma hiperferritinémia (>300 μg/L) previa presença de ferro na MO. Para a avaliação das reservas de ferro nos doentes com valores de ferritinémia dentro dos valores de referência (valores denominados pelos autores de "*indeterminados*") recorreram aos níveis de sTfR (*cut-off* 28,1 nmol/L – 2,07 mg/L). Este algoritmo sequencial apresentou uma sensibilidade de 67% (comparável com a sensibilidade do uso isolado do sTfR) e uma especificidade de 93% (comparável com a ferritinémia, o teste isolado mais específico). Entre as ferritinémias "indeterminadas", o sTfR identificou correctamente 34 dos 45 doentes (6 dos 10 com sideropenia e 28 dos 35 com reservas de ferro na MO). Como foi notado pelos próprios autores, apenas 7% dos participantes neste estudo foram submetidos a AMO com objectivo primário de caracterização da anemia, uma potencial fonte de viés, já que uma doença neoplásica (um dos motivos para AMO) pode cursar com aumento do sTfR, na ausência de AS.

Suominen et al. (38), no ano 2000, estudaram um grupo de 30 doente com artrite reumatóide (AR) e anemia concomitante. Todos os participantes foram submetidos a AMO para determinação da etiologia da anemia. De acordo com o protocolo de estudo,

os participantes que apresentavam ausência de ferro na MO (13 doentes) foram suplementados com ferro durante 16 semanas (11 doentes responderam à terapêutica, 1 não respondeu e 1 deixou o ensaio); os que apresentavam níveis de ferro baixos ou normais-baixos (6 doentes) fizeram uma suplementação durante 12 semanas (4 com resposta – classificados como deficiência funcional de ferro (DFF) – e 2 sem resposta); os doentes com reservas normais de ferro não fizeram suplementação. Comparando os doentes que responderam à terapêutica de suplementação com os doentes sem resposta à terapêutica ou não suplementados, os autores verificaram que os valores do sTfR (por imunoturbidimetria, com um *cut-off* de 2,3 mg/L) e do índice sTfR-F (*cut-off* 1,35) permitiam uma excelente discriminação entre os dois grupos. Concluíram que estes marcadores são úteis na diferenciação entre AS e ADC. O desenho deste trabalho, com a sua classificação dos doentes relativamente a resposta terapêutica apresenta-se como uma abordagem diferente para avaliação da utilidade clínica do sTfR.

Também em 2000, Ruivard et al. (39) avaliaram o valor diagnóstico do sTfR (por ELISA) relativamente às reservas de ferro em doentes com e sem anemia. Os doentes foram classificados em AS (20 doentes) e controlos (33 não-sideropénicos) com base na avaliação do AMO. Para os autores, a razão sTfR/Ferritina revelou-se o melhor marcador de sideropenia, com uma sensibilidade de 81% e especificidade de 97%. O uso isolado da ferritinémia (*cut-off*: 60 μg/L) obteve uma especificidade semelhante, mas uma sensibilidade mais baixa (76%). A especificidade para o sTfR (para um *cut-off* de 800 U/L, sem referência de qualquer factor de conversão para mg/L) foi de 67% e a sensibilidade de 62%. O índice sTfR-F não foi calculado. Os autores concluíram que, apesar da ferritinémia permanecer como o principal marcador de sideropenia, o sTfR e a sua razão com a ferritina devem ser utilizados quando a interpretação da ferritina isolada não for possível.

Em 2002 foram publicados 4 artigos, com resultados discordantes. Joosten et al. (40) estudaram 83 idosos anémicos, hospitalizados, submetidos a AMO. Os participantes foram classificados em dois grupos, baseando-se nos doseamentos de ferro na MO e na informação clínica: AS (n=34) e ADC (n=48). Os autores calcularam, para o sTfR (com um *cut-off* de 28,1 nmol/L – 2,07 mg/L, – doseado por ELISA), uma sensibilidade de 68% e especificidade de 61%, em contraste com a ferritina (*cut-off* de 50 μg/L) que registou valores de 95% e 94%, respectivamente, concluindo que a ferritina tinha maior utilidade que o sTfR no diagnóstico diferencial de AS e ADC numa população idosa.

Rimon et al. (41) também se concentraram em doentes idosos hospitalizados (n=49), com anemia sideropénica (comprovada por AMO) e um grupo de 14 controlos com reservas de ferro na MO, doseando o sTfR (por EIA) e calculando o índice sTfR-F. O uso de marcadores séricos "clássicos" (ferro sérico, saturação da transferrina e ferritina) apenas identificou 8 dos 49 doentes com AS, enquanto que o recurso ao índice sTfR-F (com um valor *cut-off* de 1,5) identificou não só esses 8 pacientes, como outros 35 – sensibilidade de 88% e especificidade de 93% (VPP de 98% e VPN de 68%). Contudo, para os autores, apesar de um índice sTfR-F aumentado, em doentes idosos com factores inflamatórios, poder estabelecer um diagnóstico de AS, um valor normal não permite afirmar a sua exclusão.

Os outros dois estudos concordaram com esta alta especificidade e sensibilidade. Fitzsimons et al. (42) numa avaliação *in vitro* da expressão membranar de TfR e o *up-take* de ferro em eritroblastos de 15 doentes com AR e AS concomitantes (AR-AS) e 12 com AR e ADC (AR-ADC), compararam, nestes doentes, os doseamentos de sTfR (sem especificação da metodologia de doseamento) com as reservas medulares de ferro (por AMO). Determinaram uma diferença estatisticamente significativa, entre os doentes com AR-ADC e AR-AS, nos valores do sTfR. Com um *cut-off* de 28,1 nmol/L (2,07

mg/L), este apresentou uma sensibilidade de 93% e uma especificidade de 92% na avaliação das reservas de ferro, comparativamente a ferritina, com um *cut-off* de 15 ou 75 μg/L, para a qual foi reportada uma sensibilidade de 15% e 62%, respectivamente, com uma especificidade de 82% (para o *cut-off* de 75 μg/L).

Finalmente, Lee et al. (30), avaliaram a utilidade do sTfR (por imunoturbidimetria) em 120 doentes com anemia (72 com AS: 15 com infecção ou inflamação e 26 com doença maligna não-hematológica concomitantes e 31 sem comorbilidades; e 48 com ADC: 23 com infecção/inflamação e 25 com doença maligna não-hematológica). Reportaram para o sTfR, com *cut-off* de 1,8 mg/L, e para a ferritina (*cut-off* de 35 μg/L) sensibilidades de 97% e 94%, respectivamente, e especificidades de 88% e 98% na detecção de sideropenia. O cálculo do sTfR-F apresentou, para os mesmos parâmetros de avaliação, valores de 100% e 98%, respectivamente. O sTfR não se mostrou superior à ferritina no diagnóstico de sideropenia. Em doentes com doença maligna o sTfR mostrou não reflectir as reservas de ferro. Os autores defendem o uso do índice sTfR-F em doentes com inflamação crónica ou infecção.

Passado um ano, Baillie et al. (5) avaliaram 40 doentes com AS (ferritinémia abaixo de 12 μg/L), 40 com ADC e 40 com AR e anemia concomitantes (seleccionados como grupo modelo de patologia inflamatória crónica). O AMO foi realizado em 20 doentes com ADC e 18 com AR (submetidos a prótese total da anca, com análise da MO da cabeça do fémur removida). O melhor marcador sérico para avaliação das reservas de ferro na MO, com uma sensibilidade de 86% e 75% (nos doentes com ADC e AR, respectivamente) e uma especificidade de 69% e 100% foi o sTfR (*cut-off* de 3,3 mg/L). Os autores concluíram que o sTfR identifica depleção das reservas de ferro, mesmo em doentes em fase aguda. Não foi calculado o índice sTfR-F. Este artigo demonstra

explicitamente a interferência da selecção dos doentes nos resultados da sensibilidade e especificidade do teste sTfR, mostrando que, numa amostra não seleccionada de doentes, a especificidade deste marcador é muito inferior àquela calculada para uma amostra altamente seleccionada.

Mais tarde, em 2005, Hanif et al. (43), em 90 doentes com ADC e 86 com AS (classificados unicamente por AMO), concluíram que o sTfR (por EIA) teve uma sensibilidade de 100% e uma especificidade de 67% no diagnóstico de AS (VPP de 75% e VPN de 100%). Os autores não apresentam quaisquer critérios de exclusão, o que pode explicar a baixa especificidade encontrada.

Em 2007, Chang et al. (44), em 49 doentes com sTfR e reservas medulares de ferro normais, 13 com níveis de sTfR aumentados e reservas medulares de ferro normais e 14 doentes sideropénicos, avaliaram a correlação entre o sTfR (por imunoturbidimetria) e as reservas de ferro na MO. Os autores concluíram que o sTfR era o marcador sérico mais sensível (100% na ausência de ferro medular, mas apenas 62% nos doentes com reservas diminuídas) na identificação da eritropoiese deficiente em ferro em doentes com patologia crónica. Apresentaram, também, um algoritmo para o diagnóstico diferencial de AS e ADC baseado no doseamento sequencial de ferritinémia e sTfR: na coexistência de inflamação (comprovada pela clínica ou por marcadores bioquímicos), uma ferritinémia <10 μg/L para mulheres ou <30 μg/L para homens seria diagnóstica de AS. Valores superiores de ferritina, após exclusão de outras causas de anemia associadas a hiperplasia eritróide, após doseamento do sTfR, diagnosticariam ADC com ou sem eritropoiese deficiente em ferro concomitante (mediante valores de sTfR

superior a 2,0 mg/L ou não, respectivamente). Este trabalho demonstra a interferência na sensibilidade do sTfR com a inclusão de doentes com reservas diminuídas no estudo.

Em 2009, Phiri et al. (45), avaliaram o sTfR (por EIA) e o índice sTfR-F, numa amostra de 381 crianças com anemia grave, todas submetidas a AMO. A sensibilidade do sTfR (cut off de 8,3 mg/L) no diagnóstico de sideropenia foi de 97% e a especificidade de 37%. Comparativamente, a ferritina (*cut-off* 30 μg/L) obteve valores de 21% e 96%, respectivamente, e o índice sTfR-F (*cut-off* de 5,6) 70% e 75%. Uma mudança do *cut-off* do sTfR para valores de 15,2 mg/L permitiu uma subida da especificidade para 76%, com consequence diminuição da sensibilidade para os 77%. Relativamente à ferritina, uma subida do *cut-off*, para os 273 μg/L, aumentou a sensibilidade para os 75%, acompanhado de uma diminuição da especificidade para os 76%. Os autores concluíram que era necessária a definição de novos valores de *cut-off* para a população em causa, não só para a ferritina como para o sTfR. Concluíram, também, que a combinação destes dois parâmetros, o índice sTfR-F, era o marcador de reservas de ferro mais robusto. No entanto, como os autores referiram, o facto de terem seleccionado casos de anemia grave, sem estudo de uma amostra controlo é um viés deste estudo.

Avaliando também o sTfR em crianças, Jain et al., em 2010 (46), estudaram 30 crianças com AS (ferritina <12 μg/L) e 30 com ADC (com PCR >20 mg/L). As crianças com ADC foram, ainda, separadas com base nos valores de sTfR (*cut-off* 3.0 mg/L). O índice sTfR-F foi calculado em todos os doentes, sendo >1,5 (*cut-off* definido pelos autores) em todos os casos de AS. Vinte e três das 30 crianças (14 com sTfR normal e 9 sTfR aumentado) foram submetidas a AMO, com avaliação das reservas medulares de ferro. O sTfR, nos doentes em que foi realizado AMO, teve uma sensibilidade de 100% e uma especificidade de 93%. Comparativamente, nos mesmos doentes, o índice sTfR-F

obteve sensibilidade e especificidade de 100%. Segundo os autores, o uso simultâneo do sTfR e da ferritina (com cálculo do índice sTfR-F) revelou-se útil no diagnóstico de sideropenia em situações de ADC. Neste estudo, apenas 23 crianças (uma amostra altamente seleccionada) com ADC foram submetidos a AMO.

No mesmo ano, Karlsson et al. (47), pretendendo avaliar a utilidade do sTfR (por imunonefelometria) numa população não seleccionada, estudaram 50 doentes idosos com anemia submetidos a AMO. Os doentes foram separados com base nos resultados do doseamento de sTfR (*cut-off* de 1,7 mg/L). A sensibilidade e a especificidade do sTfR foram de 87% e de 74%, respectivamente. Comparativamente, a ferritina, com *cut-off* de 20 μg/L para os homens e 7 μg/L para as mulheres, obteve uma sensibilidade baixa (35%) mas uma excelente especificidade (100%). O aumento do *cut-off* da ferritina para valores de 40 μg/L, representaram um aumento da sensibilidade para os 100%, com perda de especificidade para os 88%. O índice sTfR-F (*cut-off* de 3,0) obteve uma excelente sensibilidade (100%), mas uma baixa especificidade (43%). Concluiu-se que o sTfR foi inferior à ferritina e ao índice sTfR na detecção de AS numa população não seleccionada de doentes idosos com anemia.

Em 2011, três novos estudos foram publicados. Berlin et al. (3), avaliaram 32 doentes hospitalizados com anemia, com níveis de sTfR (por imunoturbidimetria) elevados (>5 mg/dL) e ferritinémias normais a elevadas (>30 μg/L). Foi feito estudo endoscópico do tracto gastrointestinal em 24 destes doentes, tendo sido detectada causa para AS em 68% destes. Num terço foi detectada uma neoplasia do tracto gastrointestinal, sem qualquer clínica ou suspeita prévia, à excepção do aumento do sTfR. Os autores concluiram que o sTfR é um bom indicador de sideropenia, recomendando o seu uso quando os valores de ferritinémia são normais ou elevados. A concepção deste estudo é

bastante diferente dos anteriores, analisando doentes que não teriam sido diagnosticados como sideropénicos com os marcadores clássicos, pelo que não teriam indicação para o estudo endoscópico que permitiu a detecção de doença neoplásica em alguns.

Novamente Karlsson, em 2011 (6), para avaliação do uso do conteúdo de hemoglobina reticulocitária (CHr) em doentes idosos com anemia, estudarou 54 doentes submetidos AMO. Foram realizados doseamentos de sTfR (por imunonefelometria) e de ferritina e calculado o índice sTfR-F. Foram comparados os doentes com AS (n=14) e os com ADC. O índice sTfR-F (*cut-off* 1.49) teve uma sensibilidade de 92% e uma especificidade de 94%. Comparativamente, o uso isolado do sTfR (*cut-off* 2,0 mg/L) ou da ferritinémia (*cut-off* 30 μg/L) obtiveram, respectivamente, sensibilidades de 86% e 87% e especificidades de 89% e 95%. O CHr não mostrou trazer qualquer informação adicional relativamente à HCM ou ao uso da ferritina ou sTfR.

Também em 2011, Skikne et al. (23) publicaram um estudo multicêntrico de avaliação do sTfR (por imunoquimioluminescência) e do índice sTfR-F no diagnóstico diferencial entre AS e ADC. Foram estudados 145 doentes com anemia. Os participantes foram diagnosticados em AS, ADC ou ADC com AS concomitante, por clínicos, baseados em critérios bioquímicos e clínicos, a quem não foram revelados os doseamentos de sTfR ou o índice de sTfR para evitar viés de diagnóstico. Valores de PCR ≥10 mg/L ou contagem de leucócitos ≥10,5x10^3/μL foram usados como marcadores de inflamação/infecção. Os doentes não foram submetidos a AMO. O índice sTfR-F foi superior ao uso isolado do sTfR ou da ferritinémia na detecção de AS, diferenciando os casos de AS com ADC dos casos de AS não-complicada. O teste isolado com maior grau de especificidade (81%), mas sensibilidade de 83%, foi o índice sTfR-F (*cut-off* de 1,03), comparativamente, a ferritina (*cut-off* 15 μg/L) teve melhor a especificidade (96%) mas menor sensibilidade (41%). A sensibilidade para o uso isolado do sTfR (*cut-*

off nos 1,55 md/L) foi de 86% com uma especificidade de 49%. Skikne propôs um algoritmo para o diagnóstico diferencial de AS, ADC e ADC com AS associada, baseado nos níveis séricos de sTfR e ferritina e cálculo do índice sTfR-F (23). Depois de excluídas outras causas de anemia, valores de ferritina ≤15 μg/L ou sTfR ≥1,55 mg/L ou um índice sTfR-F ≥1,03 apontariam no sentido de AS ou de combinação de ADC e AS (a diferenciar pela existência ou não de evidência clínicas ou bioquímicas de infecção/inflamação). Os valores de ferritina >15 μg/L, com sTfR <1,55 mg/L e índice sTfR-F <1,03, favoreceriam o diagnóstico de ADC (sem AS concomitante) ou de outra anemia não-sideropénica. O uso deste algoritmo permitiu uma sensibilidade de 92%, baixando, no entanto, a especificidade para 49% na amostra estudada. A baixa especificidade deste algoritmo (49%) levaria a investigações adicionais (muitas vezes necessárias perante um diagnóstico de sideropenia) num maior número de doentes o que levaria a eventuais custos e riscos. Os participantes neste estudo não foram submetidos a AMO, já que, considera Skikne, a grande correlação entre o sTfR e as reservas de ferro tornam desnecessário o AMO. Pode estar aqui uma fonte de viés deste estudo.

Como referido por Koulaouzidis (7), os trabalhos publicados sobre a utilidade clínica do sTfR no diagnóstico de AS são muito heterogéneos, relativamente à amostra estudada e aos critérios de exclusão (Tabela 1) e na definição do *cut-off* para o sTfR e, consequentemente, do índice sTfR-F (Tabela 2), assim como no desenho dos estudos. Uma vez que o AMO não é realizado como parte da investigação de rotina de AS ou ADC, a maior parte dos estudos existentes são baseados num baixo número de participantes ou em doentes submetidos a AMO com outros objectivos diagnósticos (que não puramente investigação de anemias), resultando em amostras altamente seleccionadas (23) que não são representativas da população clínica da prática diária.

Estudo		Ferritina			sTfR			Índice sTfR-F		
		Cut-off (µg/L)	S (%)	E (%)	*Cut-off* (mg/L)**	S (%)	E (%)	*Cut-off*	S (%)	E (%)
Punnonen (1997)		41	91	98	2,7	94	94	1,5	98	100
Junca (1998)		*nd*	*nd*	*nd*	4,5	50	74	*nd*	*nd*	*nd*
Mast (1998)		12	20	98	2,8	100	86	*nd*	*nd*	*nd*
		30	100	98						
Means (1999)		10/22*	25	99	2,07/2,2 ***	71	74	*nd*	*nd*	*nd*
Ruivard (2000)		20/30*	52	100	800 U/L	62	67	*nd*	*nd*	*nd*
		60	76	97						
Joosten (2002)		50	95	94	2,07	68	61	*nd*	*nd*	*nd*
Rimon (2002)		*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	1,5	88	93
Fitzsimons (2002)		15	15	*nd*	2,07	93	92	*nd*	*nd*	*nd*
		75	62	82						
Lee (2002)		35	94	98	1,8	97	88	1,36	100	98
Baillie (2003)	Grupo ADC	12	0	100	3,3	86	69	*nd*	*nd*	*nd*
	Grupo AR		0	100		75	100			
Hanif (2005)		*nd*	*nd*	*nd*	3,3	100	67	*nd*	*nd*	*nd*
Chang (2007)	Sem ferro na MO	100	83	79	2,0	100	79	2,5	50	100
	Ferro baixo na MO		88			62			25	
Phiri (2009)		30	21	96	8,3	97	37	5,6	70	75
Karlsson (2010)		7/20*	35	100	1,7	87	74	3,0	100	43
		40	100	88						
Jain (2010)		*nd*	*nd*	*nd*	3,0	100	93	1,5	100	100
Karlsson (2011)		30	87	95	2,0	86	89	1,49	92	94
Skikne (2011)		15	41	96	1,55	86	49	1,03	81	83
		30	59	93						

Tabela 2. Resultados de sensibilidade (S) e especificidade (E), da ferritina, do sTfR e do índice sTfR-F para diagnóstico de anemia sideropénica. **cut-off* para sexo feminino/masculino; ** foi usado um factor de 0,0738 na conversão de nmol/L para mg/L; ****cut-off* para não-afro-americanos/afro-americanos; ADC: anemia das doenças crónicas; AR: artrite reumatóide; *nd*: não descrito ou não aplicável ao estudo

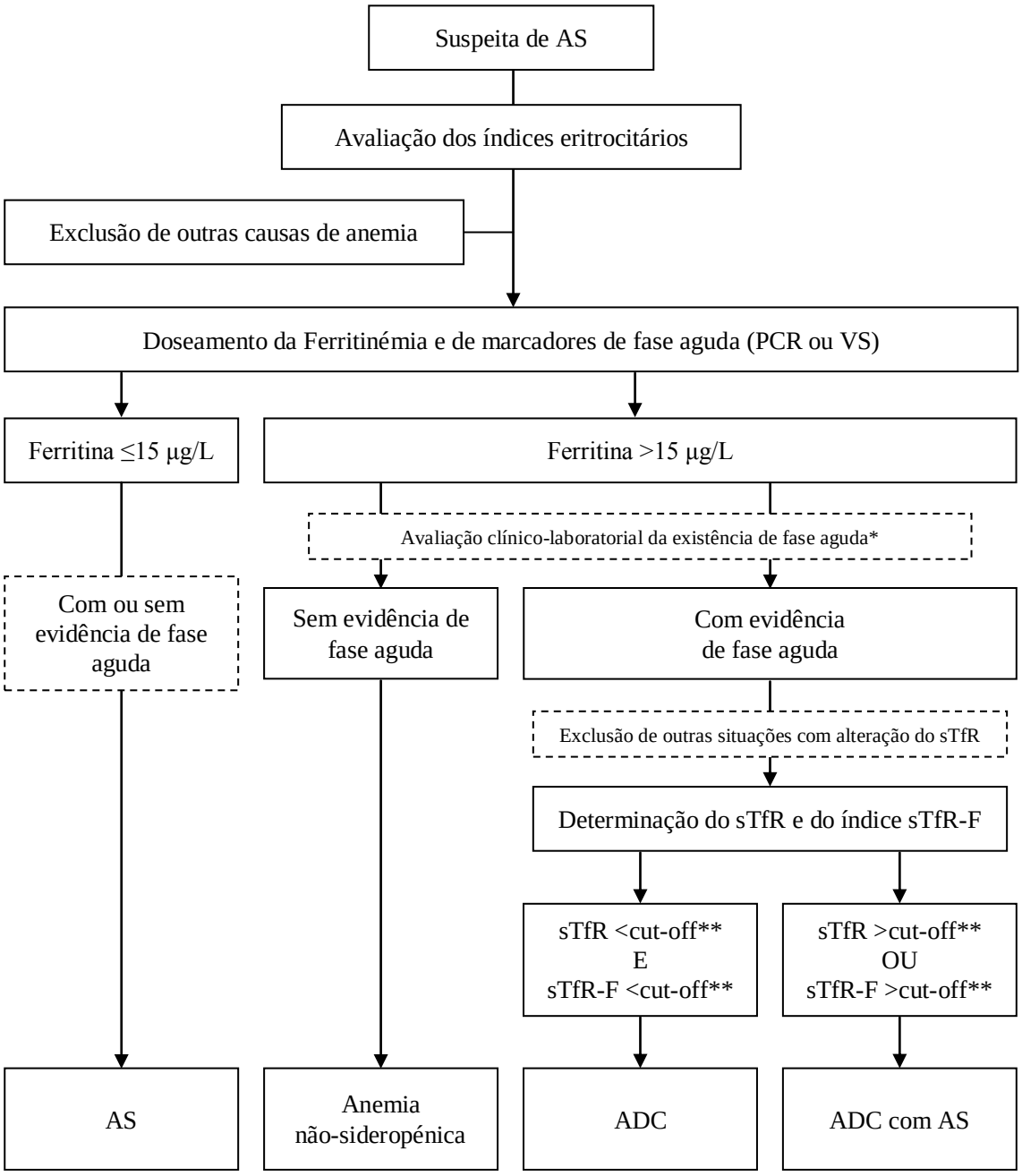
Partindo dos algoritmos anteriormente apresentados, apresentamos uma sugestão para um algoritmo de investigação de AS (Figura 1).

Uma vez que, fora de fase aguda, a ferritina é um bom indicador das reservas corporais de ferro, esta deve constituir o primeiro teste na investigação inicial de uma sideropenia, com doseamento, simultâneo, de um marcador de fase aguda (PCR ou VS). Na ausência, clínica e laboratorial, de infecção/inflamação, os valores de ferritinémia são indicativos das reservas corporais de ferro. Perante evidência clínica ou laboratorial de infecção/inflamação, deve ser realizado o doseamento de sTfR e calculado o índice sTfR-F. Elevações do sTfR ou do índice sTfR-F, após exclusão de outras causas de aumentos, são indicativos da existência de sideropenia. Valores normais destes dois marcadores, nestas situações, sugerem um diagnóstico de anemia não-sideropénica.

Contudo, em situações de alta suspeita clínica de sideropenia, não suportada pelos marcadores laboratoriais, dever-se-á, se necessário, recorrer ao AMO para determinação das reservas de ferro. Este algoritmo carece, contudo, de validação em ensaios ou na prática clínica.

CONCLUSÃO

Na ausência de inflamação ou infecção a ferritina é um bom marcador das reservas de ferro, sendo níveis séricos abaixo de 15 μg/L preditivos de deplecção das reservas de ferro, mesmo na presença de patologias concomitantes. Nestas situações o uso do sTfR traz pouca ou nenhuma informação adicional. O sTfR não se revela um marcador laboratorial a ser usado isoladamente na investigação de sideropenia, não devendo, portanto, ser utilizado como alternativa à ferritina. No entanto, a associação da ferritina e do sTfR, com o cálculo do índice sTfR-F, pode melhorar a sensibilidade do uso de qualquer um dos marcadores, isoladamente, diagnóstico de AS.



(Adaptado a partir dos algoritmos de Chang, 2007 e Skikne, 2011)

Figura 1. Proposta de algoritmo de investigação de suspeitas de anemia sideropénica. AS: anemia sideropénica; PCR: proteína C reactiva; VS: velocidade de sedimentação eritrocitária; sTfR: receptor solúvel da transferrina; índice sTfR-F: índice entre o sTfR e o logFerritina; ADC: anemia das doenças crónicas; *baseado em critérios clínicos e nos marcadores de fase aguda; **pela inexistência de padronização optamos por não indicar nenhum valor *cut-off* específico, devendo este ser definido por cada laboratório, com base no teste e na população a estudar.

Como defendido por grande parte dos autores, sustentado pela elevada sensibilidade deste índice em doentes com infecção ou inflamação concomitantes com anemia (comparativamente aos outros marcadores séricos), este será útil na distinção entre AS e ADC quando os valores de ferritinémia estão normais ou aumentados pela resposta de fase aguda.

No entanto, como o aumento dos níveis do sTfR não é exclusivo de situações de sideropenia, a história clínica do doente deve ser sempre tida em conta ao interpretar os valores do sTfR.

A existência de ensaios completamente automatizados, adaptáveis a equipamentos usados em laboratórios clínicos permite um uso generalizado do sTfR (23). No entanto, para este uso mais generalizado na prática clínica, é necessária uma padronização internacional das metodologias de doseamento do sTfR, que permita a realização de estudos multicêntricos para definição de um *cut-off* de modo a que possa ser utilizado com aquela finalidade clínica.

Um grupo de estudo internacional, sob a tutela da Organização Mundial de Saúde (OMS), tem estudado a possibilidade do uso de um padrão de referência único que permita uma padronização internacional (48). Enquanto não for conseguida esta estandardização, os valores de *cut-off* para o sTfR e, consequentemente, para o índice sTfR-F, devem ser determinados para cada um dos métodos (16).

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ANEXO

REVISTA: **Clinical Chemistry**

Author(s):
American Association of Clinical Chemists.
American Association for Clinical Chemistry.
NLM Title Abbreviation:
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ISO Abbreviation:
Clin. Chem.
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Frequency:
Twelve no. a year
Country of Publication:
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Information for Authors

Revised June 2012

Overview

Clinical Chemistry, issued monthly, is published in print and electronically by the American Association for Clinical Chemistry. The journal welcomes contributions, either experimental or theoretical, in the field of laboratory medicine. It is the leading forum for peer-reviewed, original research on innovative practices in today's clinical laboratory. In addition to being the most cited journal in the field, *Clinical Chemistry* has the highest Impact Factor among journals of clinical chemistry, clinical (or anatomic) pathology, analytical chemistry, and the subspecialties, such as transfusion medicine and clinical microbiology.

Submissions of the following nature are welcomed:

- Basic materials or principles
- Analytical techniques
- Molecular diagnostics
- Test utilization or testing-related health or financial outcomes
- Instrumentation
- Data processing
- Statistical analyses of data
- Clinical investigations in which laboratory testing has played a major role
- Laboratory animal studies of chemically oriented problems of human disease

Contributions should consist of subject matter that is original and significantly advances the state of knowledge of clinical chemistry, and conclusions that are justified from the design of the experiments and the data presented. The information must be sufficiently detailed to permit replication of the work by a competent worker in the field. Lastly, the writing must be clear, concise, and grammatically correct.

Equal consideration is given to original manuscripts in English from any country, regardless of membership in the Association. It is, however, advised that all non-English speaking authors enlist the aid of a native-English speaking colleague to correct English language usage before submission. Submissions must adhere to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (1).

Submissions are accepted via the manuscript tracking system at http://submit.clinchem.org. The "Information for Authors" will offer assistance with journal style and requirements. Please contact the Editorial Office via e-mail should you have any questions or need assistance: clinchemed@clinchem.aacc.org.

References:

1. International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. Ann Intern Med 1997;126:36-47. [Full Text]

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Standards for Reporting Scientific Data

Description of Analytical Methods and Results

Manuscripts describing the development and evaluation of the performance of methods and instruments should discuss linearity, imprecision, analytical specificity, recovery, lower limit of detection, comparability with other analytical methods, lower limit of quantification and reference interval(s). Some clinical data are usually needed.

Document the analytical advantages of the new or modified method over existing methods.

Analytical method validations should conform to the protocols and requirements in the Center for Drug Evaluation and Research (CDER) Guidance for Industry: Bioanalytical Method Evaluation, 2001 (1).

Calibration curves and linearity: Data for these studies should be analyzed by linear regression analysis (if a linear response is obtained) and should include the slope, intercept, r^2, standard deviation of residuals, and the standard deviations of the slope and intercept.

Standard deviations of repeated points may be included.

In preparing nonlinear calibration curves, authors may use any objective, statistically valid method but must specify the method used (see, e.g., (2)).

Imprecision: Studies must include estimates of "within-run" and "total" standard deviations (2). Each should be determined at low, normal, and above-normal concentrations with use of specimens that are in an appropriate biological matrix.

One method for estimating both within-run and total standard deviations is the analysis of variance experiment described in NCCLS EP5-T (3), which calls for two replicates per specimen per run and two runs per day for 20 days. This permits separate estimation of between-day and between-run, within-day standard deviations, as well as within-run and total standard deviations.

For acceptable alternatives that include only one run per day, see the cited document.

Indicators of Accuracy ("Trueness"): Accuracy (or “trueness” in the recent nomenclature) of a new method can be estimated by (a) analyses of certified Reference Materials by the new method or (b) comparisons of results of a new method with results of a Reference Method. These are the only accepted approaches to trueness. When neither is available, other evidence relevant to the ability of the method to measure the analyte (measurand) is needed. Recovery studies involve analyses after known amounts of analyte are added to the biological fluid on which the determination will be performed. Recovery of added analyte should be calculated [(final concentration – initial concentration)/added concentration], not the observed final concentration as a proportion of expected final concentration.

Interference studies should be performed to assess the effects of common interferents, including lipid particles, hemoglobin, bilirubin, and components of uremic plasma. Exogenous materials, such as ingredients of blood collection containers (tubes) and commonly used or commonly coadministered drugs that might interfere with the determination, should also be tested for interferences. Selection of materials to test should be guided by an understanding of the chemistry and physics of the measuring system. Thus chemicals that are structurally similar to the analyte should be tested to assess the selectivity of the method. (The term “selectivity” is preferred over specificity; selectivity can be quantified.) In characterizing non-spectrophotometric methods, chemicals that may interfere in the detection system should be studied more intensively than chemicals that are historically important for interference in spectrophotometric methods.

Comparison-of-methods studies should compare results by the new or proposed method with those by a reference-quality method or other generally accepted analytical method for which assay performance is documented (4, 5).

It is desirable to test 100 to 200 different samples from patients who have been selected to include a wide variety of pathologic conditions and to present a range of values for the analyte that includes those likely to be encountered in routine application.

For a table of the required number of samples, see Linnet (6).

If regression analysis is used for statistical evaluation of the data, supply slopes and intercepts (and their standard deviations) and standard deviations of residuals (Sy|x, often called standard errors of estimates). Unbiased (e.g., Deming) regression is typically required (7). A program to perform Deming regression is available online as a supplement from this journal (8).

The correlation coefficient has limited utility. Residuals plots [e.g., Bland-Altman (9, 10)] are often useful. On the horizontal axis, plot the mean of results by the two studied methods, not the result of one method.

Analytical sensitivity and detection limit: These terms are commonly confused. The International Union of Pure and Applied Chemistry defines analytical sensitivity as the ability of an analytical procedure to produce a change in signal for a defined change of the quantity. This is often visualized as the slope of the calibration curve.

The limit of detection (LOD) is defined as the lowest concentration or amount of an analyte that can be reliably identified as being qualitatively present in the sample. The limit of quantification (LOQ) is defined as the lowest concentration or amount of analyte that can be reproducibly quantified in a sample. The most acceptable criteria for ascertaining the LOQ is the concentration of analyte that can be measured with an imprecision of <20% and a deviation from target of ><20% (1). The operational definition of the LOD and LOQ must be supplied by the author. Additional considerations related to this topic are presented by Linnet (11).

Analytical quality: Results obtained for the performance characteristics should be compared objectively with well-documented quality specifications, e.g., published data on the state-of-the-art performance required by regulatory bodies such as CLIA 88, or recommendations documented by expert professional groups (12).

Reference interval (normal range): Depending on the conclusions of the accuracy studies, modification of an accepted reference interval may be indicated. Description of the reference interval study should include details about sampling; selection of subjects, including their number, age, and sex distribution; the statistical method for summarizing the results (13); and other factors that would influence the values obtained.

Mass spectrometric assays must be evaluated for matrix effects (ion suppression or enhancement) (14, 15).

Chromatograms: Chromatograms from gas-liquid and liquid chromatography should usually be presented so that readers can see the efficiency of the separation and observe the resolution from interferents in the matrix. Similar images are often needed for electrophoretic separations.

Enzyme activities: Enzyme activities may be expressed in international units (U) or katals. Temperature and other key assay features must be described in the text or by reference to a published method.

When first mentioned in the text, enzymes (whether measured by activity or mass assays) must be numbered (EC no.) in accordance with the recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the Nomenclature and Classification of Enzymes (16).

References:

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2. Linnet K, Boyd JC. Selection and analytical evaluation of methods – with statistical techniques. In: Burtis CA, Ashwood ER, Bruns DE. Tietz textbook of clinical chemistry and molecular diagnostics. 4th ed. St. Louis: Saunders, 2006: 353-407.
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16. International Union of Biochemistry and Molecular Biology, Nomenclature Committee. Enzyme nomenclature 1992. San Diego: Academic Press, 1992:862pp.

Statistics

Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results.

When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty.

Avoid sole reliance on statistical hypothesis testing, such as the use of P values, which fails to convey important quantitative information.

When appropriate, confidence intervals should be presented; see, e.g., Harris (1), Henderson (2), and references therein.

References:

1. Harris EK. On P values and confidence intervals (why can't we P with more confidence?) [Editorial]. Clin Chem 1993;39:927-8. [Full Text]
2. Henderson AR. Chemistry with confidence: should Clinical Chemistry require confidence intervals for analytical and other data? [Opinion]. Clin Chem 1993;39:929-35. [Abstract/Full Text]

Studies with Human Subjects

Authors are responsible for ensuring compliance of human studies with the Helsinki Declaration of 1975 as revised in 2008: http://www.wma.net/en/30publications/10policies/b3/index.html. Approval by the appropriate institutional committee on human research (Institutional Review Board) must be documented in the manuscript and, unless excepted by that committee, informed consent of all participants studied for the report must be included.

Animal Studies

Authors are responsible for ensuring that studies of animals are in accordance with the Committee on Care and Use of Laboratory Animals. The information can be found in the Guide for the care and use of laboratory animals. Washington, DC: Institute of Laboratory Animal Resources, National Research Council, 1985:83pp.

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Checklist for the Description of Sequence Variants at the Human Genome Variation Society

Requirements for the description of sequence variants can be found at http://www.hgvs.org/mutnomen/checklist.html.

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Tools for Diagnostic Accuracy

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- Outcomes Studies (CONSORT)
- Minimum Information about a Microarray Experiment (MIAME)
- Minimum Information about Quantitative Real-Time PCR Experiments (MIQE)

Studies of Diagnostic Accuracy (STARD)

STARD Checklist [PDF]
STARD Flowchart [PDF]

Explanatory document, with examples: http://www.clinchem.org/cgi/content/full/49/1/7

STARD guidelines: http://www.clinchem.org/cgi/content/full/49/1/1

For studies of diagnostic accuracy of tests, complete the STARD Checklist for Evaluations of Diagnostic Accuracy (1) electronically upon submission. Do not send the checklist via e-mail or upload it as supplemental material.

The STARD statement (1) and explanatory document (2) provide guidance helping authors to modify their manuscript as needed to provide the requested information. Guidelines include:

-Provide literature reference(s) describing the evaluated test(s) and criterion "gold standard" test(s) or include detailed descriptions of them.

-Follow accepted methodologic standards including the following:

a. Specify spectrum of evaluated patients (age and sex distributions, eligibility criteria, and summary of symptoms or disease stage).
b. Analyze pertinent subgroups of subjects (e.g., symptomatic and asymptomatic patients).
c. Avoid verification bias (usually by application of a "gold-standard" test to all subjects rather than to a clinically selected subset).
d. Categorize test results and patients independently to avoid reviewer bias (usually by performance of tests with blinding to patient information and vice versa).
e. Provide confidence intervals (or SE) for indices of diagnostic accuracy such as sensitivity/specificity, likelihood ratios, and areas under receiver-operating characteristic (ROC) curves (3).
f. Indicate the number of indeterminate test results and their use (if any) in further data analysis.
g. Provide laboratory data on analytical imprecision of the test (usually day-to-day CV at two or more concentrations) or reproducibility of observer interpretation (e.g., for a visually read, dichotomous [positive/negative] test).

-A flow diagram is strongly recommended (1, 2).

-When evaluating diagnostic accuracy in clinical studies, simple testing of the significance of differences between mean values of patient groups (e.g., by Student's t-test) provides insufficient information to assess diagnostic accuracy.

-Scatter plots of data, calculations of diagnostic sensitivities and specificities and their confidence intervals (3), and use of approaches such as ROC curves (4), cumulative distribution analyses (5), likelihood ratios (6), and discriminant analysis (7) provide information that is appropriate to specific situations.

-Confidence intervals should be provided (1).

-Discussions of predictive values in illustrative settings may be useful additions to assess the potential clinical utility of tests.

-Analysis of serial measurements requires special attention (8).

References:

1. STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD statement. Clin Chem 2003;49:1-6. [Abstract/Full Text]
2. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. Clin Chem 2003;49:7-18. [Abstract/Full Text]

3. Harper R, Reeves B. Reporting of precision of estimates for diagnostic accuracy: a review. BMJ 1999;318:1322-3. [Full Text]
4. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine [Review]. Note that in Figs. 4-12 in this paper, the labels for the x-axis at the top and bottom are reversed. The (correct) dual labeling of the x-axis solves the problem of whether to plot specificity or 1 - specificity on the x-axis. Clin Chem 1993;39:561-77. [Abstract/Full Text]
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6. Albert A. On the use and computation of likelihood ratios in Clinical Chemistry. Clin Chem 1982;28:1113-9. [Abstract/Full Text]
7. Solberg HE. Discriminant analysis. Crit Rev Clin Lab Sci 1978;9:209-42.
8. Matthews JNS, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. Br Med J 1990;300:230-5.

Outcomes Studies (CONSORT)

Outcomes studies (CONSORT)
CONSORT Checklist [PDF]
CONSORT Flowchart [PDF]

The CONSORT statement (1), although designed for randomized controlled trials and used for therapeutic rather than diagnostic interventions, is recommended as an often-useful guide.

For questions, contact *Clinical Chemistry* via e-mail at clinchemed@clinchem.aacc.org.

References:

1. Moher D, Schulz KF, Altman DG for the CONSORT Group. The CONSORT statement: revised recommendations for improving the quality of reports of parallel group randomized trials. www.consort-statement.org.

Minimum Information about a Microarray Experiment (MIAME)

MIAME Checklist [PDF]

MIAME describes the minimum information about a microarray experiment that is needed to enable interpretation of the results of the experiment unambiguously and potentially to reproduce the experiment.

The MIAME checklist is a description of MIAME principles designed to help authors, reviewers, and editors of scientific journals meet MIAME requirements and to make microarray data available to the community in a useful way.

MIAME is neither a dogma nor a legal document - it assumes a cooperative data provider and a fair reviewer.

Other MIAME extensions can be found at the following website: http://www.mged.org/Workgroups/MIAME/miame.html

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Minimum Information about Quantitative Real-Time PCR Experiments (MIQE)

MIQE Checklist [PDF]

For studies that include quantitative real-time PCR experiments, complete the MIQE checklist for evaluation of qPCR experiments during electronic submission. Do not send the checklist via e-mail or upload it as supplemental material. The full text of the MIQE guidelines is available online: http://www.clinchem.org/cgi/content/short/55/4/611. A PDF version for all essential components of the checklist can be obtained by the link above.

Recommended nomenclature should be used, including:

- **qPCR** for quantitative real-time PCR
- **RT-qPCR** for reverse transcription quantitative real-time PCR
- **reference genes** instead of housekeeping genes
- **hydrolysis probes** instead of TaqMan® probes
- **dual hybridization probes** for HybProbes® (LightCycler®) probes
- **quantification** instead of quantitation
- **Cq** instead of Ct, Cp, or TOP
- **quantification cycle** instead of threshold cycle or crossing point

Authors are also encouraged, but not required, to include the additional desirable items of the MIQE guidelines (1). The MIQE guidelines are intended to help authors plan, perform and present qPCR experiments. They are also a guide for reviewers and editors to judge the quality of qPCR data. Incomplete information may be grounds for manuscript rejection. Use of Supplemental Data is encouraged as necessary. The most common errors in performing and reporting qPCR data include:

a. Not enough information for others to replicate the experiment, including how the nucleic acid was prepared, reverse transcribed, and amplified. Primer sequences are required. Probe sequences are strongly encouraged especially in methods manuscripts, but their omission may be acceptable in clinical manuscripts if they are commercially available as products.
b. Inadequate storage and/or nucleic acid preparation, leading to poor nucleic acid quality and variable results.
c. Suboptimal primers for reverse transcription and/or PCR resulting in low yield, specificity and/or PCR efficiency.
d. Inappropriate analysis of data.
e. Use of a single reference gene in RT-qPCR without justification (2).

The guidelines require not only delineation of what was done, but presentation of evidence that validates the method used. For example, these include evidence of RNA integrity and purity, PCR specificity, calibration curves and calculations of PCR efficiency and limits of detection. At the option of the editor, MIQE requirements

may be relaxed in reports using qPCR arrays, although all manuscripts will be judged on their relative merit, and the relative merit of a manuscript using qPCR increases as compliance with the MIQE guidelines increases.

References:

1. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009 Apr;55(4):611-22.
2. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 2002 Jun 18;3(7):RESEARCH0034.

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- Inspiring Minds
- Letter to the Editor / Reply
- Mini-Review
- Obituary
- Opinion
- Perspective
- Point/Counterpoint
- Q&A
- *Quo Vadis?*
- Reflection
- Review
- Special Report
- Unveiling the Right Side
- What Is Your Guess?

Article

Research or scientific articles are submitted directly from authors. There are no restrictions on topics that are considered for publication, as long as the subject matter is original and relates experimentally or theoretically to the field of laboratory medicine. The information must be sufficiently detailed not only to enable readers to understand and appreciate the material presented, but also to permit replication of the work by other scientists in the field.

Articles should contain a structured abstract limited to 250 words and formatted to include separate headings of: Background, Methods, Results, and Conclusions. The main text should not exceed 3,500 words. The manuscript should have no more than 40 references and a total of 6 tables and/or figures. Supplemental data are permitted for Articles.

Articles should list no more than 15 authors, with any additional contributors listed in the Acknowledgments. Although exceptions are rare, you may email clinchemed@clinchem.aacc.org detailing each author's contribution to your submission, which will be forwarded to the editor.

Authors may submit short videos to complement their Articles. Videos can be used to illustrate a laboratory technique, hardware prototype, or clinical presentation that would benefit from such an addition, or to visually communicate to the reader novel features, special steps in a procedure, pitfalls, or other information that may not be easily conveyed through text or a figure. Videos should be of high quality, no more than 5 minutes in length, and submitted as .mp4 files. Please do not send proprietary file types such as .wmv (Windows Media) and .rm (Real Media) files.

Authors of Articles will be prompted at submission to provide a brief summary of their work, not to exceed 100 words. In the event of acceptance, this will be recorded and included as an audio file in all online versions of the table of contents. The Audio Summary should address the following questions:

1. What was the paper about/what was the rationale for the study?
2. How did you approach the problem?
3. What were your findings?
4. What are the implications of the findings and how do they add to the field?

A simple phonetic spelling of the first author's full name should also be provided.

Brief Communication

Brief Communications are submitted directly from authors. They describe original research from studies that may not be as comprehensive in nature as full Articles, but have sufficient originality and utility to be considered for publication. The information must be sufficiently detailed so that readers can understand and appreciate the material presented. The figure and/or table should be concise and limited in scope.

Brief Communications should contain a structured abstract limited to 250 words and formatted to include separate headings of: Background, Methods, Results, and Conclusions. The main text should not exceed 1,500 words. The manuscript should have no more than 20 references and a total of 1 table and 1 figure. Multipart figures are not permitted in Brief Communications. Rare exceptions are made. Supplemental data are permitted for Brief Communications.

Brief Communications should list no more than 15 authors, with any additional contributors listed in the Acknowledgments. Although exceptions are rare, you may email clinchemed@clinchem.aacc.org detailing each author's contribution to your submission, which will be forwarded to the editor.

Citation Classic

Citation Classics are typically invited submissions that highlight a landmark article in the field of clinical chemistry. In this feature, one of the authors of the original article provides some historical insights and anecdotal stories surrounding its publication.

Citation Classics should not include an abstract and are limited to 600 words and no more than 6 references. Generally, tables and figures are not permitted; however, if the text does not exceed 500 words, one table or figure will be allowed. Also, supplemental data are not permitted for Citation Classics.

Clinical Chemist

This monthly feature provides a forum for informing readers about general items of interest. Topics might include announcements for upcoming conferences, awards received by members of the AACC, announcements of new features in the journal, humorous items, artwork or photographs from readers, or general scientific news. Readers may submit items for consideration in the following categories: Unveiling the Right Side and What Is Your Guess?, following the specific guidelines for each. The editors will make the final decision on the appropriateness and priority for inclusion in this section of the journal.

Clinical Case Study / Commentary

Clinical Case Studies are submitted directly from authors. These articles are intended to be educational, with the goal of helping to develop or improve problem-solving skills. Clinical Case Studies may report unusual (although not necessarily rare) biochemical manifestations of disease, atypical presentation of disease, situations where the laboratory helped in making or clarifying a diagnosis, or information that would be helpful in understanding the pathophysiology of a disease.

Two accompanying commentaries will introduce additional concepts that may be useful to readers, discuss confounding factors that might affect a diagnosis or analytical result, provide comments about the case itself, or direct the reader to additional resources on the topic. Commentaries are invited and authored by clinical chemists, physicians, or scientists with expertise in the area.

A Clinical Case Study should not include an abstract. It should, however, include a case description of no more than 500 words followed by the text, which is limited to 1,000 words. References are limited to 10, and the tables and figures are limited to 2 in total. Authors should include 3-5 brief questions regarding the case that would stimulate discussion and learning about the disease state. These questions will be circulated to educational centers before publication. The author should also list up to 5 points to remember at the end of the manuscript. The questions and points are not included in the manuscript count of 1,500 words. Supplemental data are not permitted for Clinical Case Studies.

Commentaries are limited to 300 words. They should not include an abstract, references, or tables and figures. Supplemental data are not permitted for Commentaries.

Correction

Corrections are unique and will be considered on a case-by-case basis. Authors are encouraged to contact the Editorial Office at clinchemed@clinchem.aacc.org should they wish to submit a Correction or should they find a printer error that needs correcting.

Editorial

These are typically invited submissions. Editorials provide opinions and observations by an expert in the field about the subject matter or content of a scientific paper published in *Clinical Chemistry*. In addition to further educating readers on a selected topic, Editorials are designed to stimulate readers to formulate their own opinions about a paper and its value to the field. In some cases, Editorials may also be independent opinions and observations about a controversial topic or changes taking place in the field.

Editorials are limited to 1,500 words. They should not include an abstract. References are limited to 15, and tables and figures are not permitted. Supplemental data are not permitted for Editorials.

Inspiring Minds

These biographical articles are commissioned to present the achievements of distinguished clinical chemists, as well as their philosophical views on their professional life and the field of clinical chemistry.

Letter to the Editor / Reply

Letters are submitted directly from authors in response to published Articles and Brief Communications only. Other types of Letters will not be considered for publication. Letters report observations on interferences, suggestions to improve test performance, or other observations that are of importance to the wider audience. A Reply to a Letter may also be solicited by the editors. The one figure or table provided should be concise and should not be multipart (i.e., Fig. 1A, 1B, 1C, Part 1, Part 2).

A Letter to the Editor is limited to 750 words. It should not include an abstract. The references are limited to 5, and only 1 table or figure is permitted. Supplemental data are not permitted for Letters to the Editor.

A Reply is limited to 750 words. It should not include an abstract. The references are limited to 5, and only 1 table or figure is permitted. Supplemental data are not permitted for Replies.

Letters to the Editor and Replies should list no more than 5 authors, with any additional contributors listed in the Acknowledgments. Although exceptions are rare, you may email clinchemed@clinchem.aacc.org detailing each author's contribution to your submission, which will be forwarded to the editor.

Mini-Review

Mini-Review articles are typically invited submissions. Mini-Reviews are intended to provide a general overview of a topic. Basic information is provided, along with selected references that can aid the reader in obtaining additional information about the subject. The use of illustrative figures or tables is encouraged.

A Mini-Review article should consist of a structured abstract limited to 250 words with headings of Background, Content, and Summary. The text should not exceed 3,500 words. The manuscript should have no more than 40 references and a total of 4 tables and/or figures. Supplemental data are permitted for Mini-Review articles.

Mini-Reviews should list no more than 15 authors, with any additional contributors listed in the Acknowledgments. Although exceptions are rare, you may email clinchemed@clinchem.aacc.org detailing each author's contribution to your submission, which will be forwarded to the editor.

Obituary

Obituary announcements and associated biographies can be commissioned by the Journal or submitted by authors. Prior to submission of an Obituary, authors should contact the Journal with information about the person who has passed away and with a description of the individual's career achievements and unique contributions. A decision will then be made on proceeding with the Obituary.

Obituaries should include personal information about the deceased (birthplace, education, place of residence, employment), highlights of this person's achievements (research accomplishments, awards, elected positions, committees, service to the profession), and anecdotal information about what made the person unique.

Obituaries are limited to 600 words with no abstract, references, or tables. One figure/image file is permitted. Supplemental data are not permitted for Obituaries.

Opinion

Opinion articles present the belief or personal view of the author(s) on a specific topic. An opinion implies a conclusion thought out yet open to dispute. Opinion articles are often a formal expression by an expert of his/her judgment or advice. Unlike Editorials and Letters to the Editor, Opinion articles do not comment on, or refer to, specific papers published in the journal.

An Opinion should not include an abstract and is limited to 1,500 words, 15 references, and 1 table or figure. Supplemental data are not permitted for Opinions.

Perspective

These articles are invited submissions. Perspectives highlight a clinical, analytical, or basic science report that was published in a journal other than *Clinical Chemistry* but has implications for the practice of clinical chemistry.

Perspectives should not include an abstract. They are limited to 1,500 words, 5 references, and 1 table or figure. Supplemental data are not permitted for Perspectives.

Point/Counterpoint

These articles are typically invited submissions from experts in a selected discipline and provide different viewpoints on a topic that may be controversial, lacks consensus in the scientific community, or may be of high public interest. In most cases an author or group of authors is asked to write the first half of the article, describing the importance of the topic, challenges to be addressed, current limitations, and/or unmet needs. A second author or group of authors is invited to provide a "Counterpoint" discussion of a different viewpoint or critical factors.

Point/Counterpoint submissions should not include an abstract. The manuscript is limited to 1,500 words, 15 references, and 1 table or figure. Supplemental data are not permitted for Point/Counterpoint.

Q&A

This invited feature is meant to highlight a timely and important issue, either clinical or analytical, through a series of questions posed to leaders in the field by a moderator. The moderator is required to include an introductory paragraph and photographs of each expert. Five to 8 questions may be posed to 3 to 5 experts with the moderator documenting the answers. Submissions are limited to 2,500 words.

Quo Vadis?

Quo Vadis? is a monthly feature in which a question will be posed to clinical chemists 40 years of age and under. Select answers will be published in the Journal, posted on the Journal and AACC websites, and shared via social networking and broadcast e-mails. Responses should be 100 words or less and should be submitted via e-mail to quovadis@aacc.org along with the full name and address and a high-resolution photograph of the responder.

Reflection

These articles are invited submissions. Reflections are authored by highly accomplished scientists in their field who have greatly contributed to science. Reflections will be reserved for special issues and will focus on the specific advancements the individual has made in his or her field.

Reflections are limited to 2,000 words and should not include an abstract. The references are limited to 20, and 1 table or figure is permitted. Supplemental data are not permitted for Reflections.

Review

Review articles are typically invited submissions. Reviews are intended to provide comprehensive coverage of a topic, including background clinical or analytical information, the relevance and importance of the subject matter, and potential future directions. The use of illustrative figures or tables is encouraged.

A Review article should consist of a structured abstract with headings of Background, Content, and Summary limited to 250 words. The text should not exceed 5,000 words. The manuscript should have no more than 75 references and a total of 6 tables and/or figures. Supplemental data are permitted for Review articles.

Reviews should list no more than 15 authors, with any additional contributors listed in the Acknowledgments. Although exceptions are rare, you may email clinchemed@clinchem.aacc.org detailing each author's contribution to your submission, which will be forwarded to the editor.

Special Report

Special Reports may be submitted directly by authors or invited by the journal. The types of papers that would be considered include consensus reports, guideline development, position statements, or evidence-based recommendations on test utilization or quality specifications. The editors may also decide to classify other miscellaneous submissions under this heading.

A Special Report should consist of a structured or unstructured abstract limited to 250 words. The main text should be no more than 5,000 words. The manuscript should have no more than 40 references and a total of 4 tables and/or figures. Supplemental data are permitted for Special Reports.

Special Reports should list no more than 15 authors, with any additional contributors listed in the Acknowledgments. Although exceptions are rare, you may email clinchemed@clinchem.aacc.org detailing each author's contribution to your submission, which will be forwarded to the editor.

Unveiling the Right Side

Submissions should highlight the creative side of someone in the field of chemistry. This can be poetry, a short story, photographs, or other creative artwork. Submissions are limited to 400 words and/or one image, photograph, or poem. All submissions are subject to review. Cover letter should state interest in contributing to Unveiling the Right Side and must be submitted under the category of Clinical Chemist.

What Is Your Guess?

Submissions for this 1-page quiz should consist of an image or lab values, a case description (less than 75 words), 3 questions, case discussion (less than 75 words), and no more than 5 references. Cover letter should state interest in contributing to What Is Your Guess? and must be submitted under the category of Clinical Chemist.

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Manuscript Preparation

Author Contribution Requirements

Manuscripts are considered for publication with the understanding of the following:

- Each author has participated significantly in the work in a substantive way and is prepared to take public responsibility for its content;
- Each listed author must have

    1. participated in conception, design, analysis, or interpretation;
    2. drafted or critically revised the manuscript; and
    3. read and approved the final submitted manuscript and revisions.

Any change in authors and/or contributors after initial submission must be approved by all authors. This applies to additions, deletions, change of order to the authors, or contributions being attributed differently.

Author limits may be imposed for certain submission types. Please review the specific requirements for your submission type. Please list only the allowed number of authors in the author list, with the remaining contributors listed in an Acknowledgment. Exceptions may be made at the discretion of the editor.

Any alterations made to the manuscript after submission must be approved by the editor. Authors may upload the request letter to the online submission system as a supplemental file or send the letter via e-mail to the *Clinical Chemistry* editorial office at clinchemed@clinchem.aacc.org. The editor may contact any of the authors and/or contributors to ascertain whether they have agreed to any alteration.

1. The International Committee of Medical Journal Editors (ICMJE) Uniform Guidelines for Manuscripts Submitted to Biomedical Journals (1) specifically state that “all contributors who do not meet the criteria for authorship, such as a person who . . . provided purely writing assistance" be named in the acknowledgments.

2. Important contributions to an article should be recognized and appropriately attributed in that article.

   Good medical writers and editors can make valuable contributions to the publication process, often improving the clarity of the communication, broadening the scope of literature review, providing an extra level of data review, adding balance and objectivity, and shortening the time needed for manuscript development.

The American Medical Writers Association (AMWA) http://www.amwa.org believes that these important contributions deserve recognition.

3. Readers benefit from knowing about the involvement of professional writers and editors.

   Disclosing the editorial contribution and the source of funding of the writer and editor allows the reader to make informed judgments about the objectivity of the article.

   Note that the AMWA position statement recommends acknowledgment of pertinent professional or financial relationships as well as acknowledgment of the contributions of writers and editors.

   It also recommends that the person being acknowledged be given the opportunity to grant or refuse permission for the acknowledgment.

References:

1. International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. Ann Intern Med 1997;126:36-47. [Full Text]

Manuscript Guidelines

- MS Word document (.doc) is required for all submissions.
- All figures must be uploaded separately as Image Files in Tagged Image File Format (.tiff), Encapsulated Postscript (.eps) or PowerPoint (.ppt) with embedded fonts.
- All submissions must be double-spaced, 1 inch margin, twelve-point font size in Arial, Helvetica, Times New Roman and Symbol font (for non-text characters).
- All submissions must be page numbered.
- Do not use headers or footers.
- Use standard abbreviations and define all nonstandard abbreviations.
- All submissions require a title page.
- Reporting of Concentration Units:

  1. Analyte concentrations will be expressed in the text in the traditional mass unit (mg/dL, ng/ml, and so forth) followed by the SI unit in parentheses. Exceptions would include those analytes in which SI units are used globally, such as electrolytes (use mmol/L for sodium, potassium, chloride, and CO_2 values), or cases in which the traditional unit and the SI unit differ by only a factor of 1000 in both the numerator and denominator (e.g., ng/mL vs µg/L). In such cases, the unit of measure consistent with common practice will be used.
  2. The unit of measure mg/L should be used only when referring to SI units or when national or international guidelines require or recommend that the concentration of an analyte be expressed in that unit of measure, such as for high-sensitivity C-reactive protein. The unit of measure U/L will be used for most enzyme activites.
  3. Only traditional units will be used for tables and figures in the printed version of a report; SI conversion factors will be provided in legends. All tables and figures will also be presented in SI units. These tables and figures will be made available in online supplements to published articles and letters. Authors will provide both versions before final acceptance of a manuscript. SI units are available at Bureau International des Poids et Mesures.

- Supplemental Data are accepted for online publication only and are limited by submission types (See Types of Submissions for details).
- Follow the guidelines for length restrictions, abstract, reference, table and figure, and supplemental data limits as outlined in the chart below:

Type of Submission	Word Limit*	Structured** (S) or Unstructured (U) Abstract:Word Limit	Maximum Number of References	Total Number of Tables/Figures	Supplemental Data Permitted
Article	3,500	S: 250	40	6	Yes
Brief Communication	1,500	S: 250	20	1 each***	Yes
Citation Classics	600	Nonapplicable	6	Nonapplicable	No
Clinical Case Studies (Case description) w/ 3-5 questions and up to 5 points to remember	1,000 (500)	Nonapplicable	10	2	No
Commentary	300	Nonapplicable	Nonapplicable	Nonapplicable	No
Editorial	1,500	Nonapplicable	15	Nonapplicable	No
Letter to the Editor / Reply	750	Nonapplicable	5	1***	No
Mini-Review	3,500	S: 250	40	4	Yes
Obituary	600	Nonapplicable	Nonapplicable	1	No
Opinion	1,500	Nonapplicable	15	1	No
Perspective	1,500	Nonapplicable	5	1	No
Point/Counterpoint	1,500	Nonapplicable	15	1	No
Reflection	2,000	Nonapplicable	20	1	No
Review	5,000	S: 250	75	6	Yes
Special Report	5,000	S or U: 250	40	4	Yes

*Word limit consists of the body of the manuscript only; it does not encompass the title page, abstract, acknowledgments, references, tables, figure legends, figures, or Clinical Case descriptions, questions, and points to remember.

**Structured abstracts contain the headings (1) BACKGROUND, (2) METHODS, (3) RESULTS, (4) CONCLUSIONS for all applicable article types except for Reviews and Mini-Reviews. Abstracts for Reviews and Mini-Reviews contain the headings (1) BACKGROUND, (2) CONTENT, (3) SUMMARY.

***If a figure accompanies the paper, the image should not be multipart (i.e., Fig. 1A, 1B, 1C, Part 1, Part 2).

Journal Categories

Articles are grouped in the journal according to subject. Upon submission, authors are required to select the journal category that best describes their manuscript from the list indicated below:

- Molecular Diagnostics and Genetics (MDG)
- Evidence-Based Laboratory Medicine and Test Utilization (TUO)
- Hemostasis and Thrombosis (HAT)
- Proteomics and Protein Markers (PPM)
- Cancer Diagnostics
- Lipids, Lipoproteins, and Cardiovascular Risk Factors (LLP)
- Drug Monitoring and Toxicology (DMT)
- Hematology (HEM)
- Endocrinology and Metabolism (END)
- Point-of-Care Testing
- Automation and Analytical Techniques (AAT)
- Informatics and Statistics
- Laboratory Management (LMA)
- General Clinical Chemistry (GCC)
- Animal Clinical Chemistry (ANI)
- Clinical Immunology (CLI)
- Pediatric Clinical Chemistry (PED)
- Nutrition (NUT)
- Infectious Disease
- Other Areas of Clinical Chemistry (OTH)

Title Page

The first page of the manuscript should include the following information:

1. full title of submission, which should include only generic, not trade, names when describing a test, assay, etc.;
2. running head of fewer than 65 characters (including spaces);
3. list of all authors (first name, middle initial, and last name, in that order);
4. names of each author's institution and an indication of each author's affiliation;
5. name, address, telephone and fax number, and e-mail address of the corresponding author;
6. keywords;
7. any previous presentation of the manuscript;
8. list of abbreviations, in order cited; and
9. list of any "Human Genes" discussed in the paper. For each gene, indicate the gene symbol and gene name approved by the HUGO Gene Nomenclature Committee. Include other name(s) that are used in the paper or are widely used in the literature for the gene.

Abstract (Structured and Unstructured)

Structured abstracts should be formatted to include separate headings of: Background, Methods, Results, and Conclusions. For Mini-Review and Review articles the headings should be: Background, Content, and Summary. Both structured and unstructured abstracts are subject to a limit of 250 words.

Unstructured abstracts do not require separate headings.

Citation Classics, Clinical Case Study, Commentary, Editorial, Inspiring Minds, Letters to the Editor, Reply, Obituary, Opinion, Perspective, and Point/Counterpoint submissions do not require an abstract.

Abstracts must be uploaded to the abstract field of the Manuscript Metadata page online upon submission as well as the manuscript.

Text

The body of the manuscript should be written as concisely as possible and must not exceed the manuscript category word limits described herein. All pages must be double-spaced and all lines numbered. The body of the paper should include: Introduction, Materials and Methods, Results and Discussion.

- Introduction - why was the study undertaken?
- Materials and Methods - how was the study done?
- Results - what did the study find?
- Discussion - what might it mean, why does it matter, what next?

Full corporate names of manufacturers of materials should be utilized (omit Inc., Co., GmbH and similar words). After the first mention, use a shorter name (e.g., for Bio-Rad Laboratories, use Bio-Rad). Only the manufacturer's name should be used, unless the item in question was a gift, in which case the city, state, and e-mail or website of the company should be included.

Reporting of Concentration Units: 1. Analyte concentrations will be expressed in the text in the traditional mass unit (mg/dL, ng/ml, and so forth) followed by the SI unit

in parentheses.

Use of human subjects requires a statement in the text indicating whether the procedures followed were approved by your institution's responsible committee or were in accordance with the current revision of the Helsinki Declaration and whether subjects gave informed consent.

Disclosures/Conflict of Interest

All authors are required to complete a full disclosure form upon submission. Please note that the form is not limited to those disclosures that constitute a potential conflict of interest. The disclosure form is electronic and completed during the submission process within the Bench>Press submission system. Disclosures should not be included in an Acknowledgment or elsewhere within the submitted manuscript file. All grants or other forms of research funding applicable to the report, as well as all relevant employment or leadership roles, consulting or advisory relationships, stock ownership, and patents occurring within the previous 24 months should be included in this form. The recipient(s) of all applicable grants or other funding must be specified. Failure to adhere to this guideline may result in a return of the submission to the author for correction.

In order to complete disclosures, register with *Clinical Chemistry* at http://submit.clinchem.org/cgi/registration using a valid e-mail address. Each author is required to be registered and must individually complete the disclosures. If you are registered under a different e-mail address from the one the submitting author has provided, you will not be able to access the disclosures. After registration, the system may take up to 15 minutes to refresh before the disclosure form appears.

Each author is expected to disclose any relevant financial relationships held personally within the last 24 months. Any companies or proprietary entities producing scientific services, which have an investment, licensing, or other commercial interest in the subject matter under consideration in the submitted manuscript, must be disclosed.

Such information is held in confidence while the manuscript is under review and does not influence the editorial decision on reports of research; upon acceptance, relevant information is added to the manuscript for publication.

Authors of editorials are expected to be free of significant financial associations with companies that may be affected by topics discussed in the manuscript and must also complete a full disclosure at the time of submission.

The American Medical Writers Association (AMWA) recognizes the valuable contributions of biomedical communicators to the publication team. Biomedical communicators who contribute substantially to the writing or editing of a manuscript should be acknowledged with their permission and with disclosure of any pertinent professional or financial relationships. In all aspects of the publication process, biomedical communicators should adhere to the AMWA code of ethics at http://www.amwa.org/default.asp?id=114.

Acknowledgments

Acknowledgments are limited to 60 words and should follow the main text of the manuscript directly above the reference section in a separate paragraph heading labeled “Acknowledgments.” They should not appear as footnotes.

Do not include financial support, or other disclosure/conflict of interest information in the Acknowledgment. This information should be included in the Author Disclosure/Conflict of Interest form. If you include research funding in the Acknowledgment, you must specify which author or authors received the funding or if the funding was given to the group or institution.

References

References should appear in a separate section directly following the body of the manuscript. The section must be labeled “References” with no additional punctuation.

- Italic or boldface type is prohibited in the referenced citations.
- List and number the references in the order that they appear in the text.
- Do not use the MS Word document (.doc) numbering tool. Number each reference manually with the numeral and a period, followed by a space.
- For articles with more than seven authors, list the first six authors followed by "et al." For seven or fewer, list all authors.
- Authors’ names are inverted (last name, first/second initial). Do not add periods or commas within an individual author name; however, separate author names with a comma and end the author list with a period (Smith J, Doe JJ, Adams B.).
- Capitalize only the first word of the title or subtitle, and any proper names that are part of the title. The title should end with a period.
- The Journal names should be abbreviated as indicated at PubMed. For a list of journal abbreviations, please visit: LinkOut Journals.
- Do not add a period after the journal abbreviation, but continue with a space followed by the year.
- The year should be followed by a semicolon and then the volume number, which is followed by a colon and then the page numbers. Delete redundant numbers, for example 1998;12:231-45.
- Do not include the months in parentheses; this information is not needed.
- Use inclusive page numbers for articles and book chapters.

Abstract and supplement numbers should be provided, if applicable. Citations of unpublished abstract books, manuscripts in preparation or under review, personal communications, and manufacturers’ information should only be cited in the text and should not appear in the reference list. Personal communications should also be listed parenthetically and should contain the first initial and last name of the contact as well as the month and year of the communication. A copy of written permission from the contact to use the communication must also be provided. Published manuscripts and manuscripts that have been accepted and are pending publication should be cited in the reference list. Note that unpublished material must be published, at least online, by the time of publication of the citing article.

In press references cited in the reference list must be accompanied by a copy of the cited manuscript and a letter of acceptance, or a complete author proof from the publisher. These resources should be uploaded as supplemental data along with the manuscript and other print materials.

The submission system will extract the references from the submitted MS Word document (.doc) to display in a hyperlinked HTML format as an aid for reviewers and editors. This linking option allows for the checking of the correct formatting and the accuracy of the citations.

Authors must check the linking of their references to PubMed during the “Ready for You to Proof” stage of submission. Correct linking of the references depends on strict adherence to Journal style as indicated.

Reference Style

- *Journal article with seven or fewer authors:*

1. Vermeersch P, Mariën G, Bossuyt X. A case of pseudoparaproteinemia on capillary zone electrophoresis caused by geloplasma. Clin Chem 2006;52:2309-11.

- *Journal article with more than seven authors:*
  2. Fiechtner M, Ramp J, England B, Knudson MA, Little RR, England JD, et al. Affinity binding assay of glycohemoglobin by two-dimensional centrifugation referenced to hemoglobin Alc. Clin Chem 1992;38:2372–9.

- *Abstract:*
  3. Hortin GL, King C, Kopp J. Quantification of rhesus monkey albumin with assays for human microalbumin [Abstract]. Clin Chem 2000;46:A140-1.

- *Editorial:*
  4. Demers LM. New biochemical marker for bone disease: is it a breakthrough? [Editorial]. Clin Chem 1992;38:2169–70.

- *Letter to the Editor:*
  5. Davey L, Naidoo L. Urinary screen for acetaminophen (paracetamol) in the presence of N-acetylcysteine [Letter]. Clin Chem 1993;39:2348–9.

- *Book Chapter:*
  6. Rifai N, Warnick GR. Lipids, lipoproteins, apolipoproteins, and other cardiovascular risk factors. In: Burtis CA, Ashwood ER, Bruns DE, eds. Tietz textbook of clinical chemistry and molecular diagnostics. 4th Ed. St. Louis: Elsevier Saunders 2006:903-81.

- *Thesis:*
  7. Haughton MA. Immunonephelometric measurement of vitamin D binding protein [MAppSci thesis]. Sydney, Australia: University of Technology, 1989:87pp.

- *Books and Monographs (serial volumes should be treated as journals):*
  8. Harrell FE Jr. Regression modeling strategies. New York: Springer; 2001.
  9. Bailar JC III, Mosteller F, eds. Medical uses of statistics, 2nd Ed. Boston: NEJM Books; 1992:449pp.

- *Published Ahead of Print:*
  10. Milbury CA, Li J, Makrigiorgos GM. PCR-based methods for the enrichment of minority alleles and mutations. [Epub ahead of print] Clin Chem February 6, 2009 as doi:10.1373/clinchem.2008.113035.

- *Supplement:*
  11. Castelli WP. Lipids, risk factors and ischaemic heart disease. Atherosclerosis 1996;124 Suppl:S1-9.

- *Internet Source:*
  12. American Association for Clinical Chemistry. AACC continuing education. http://www.aacc.org/development/ce/pages/default.aspx# (Accessed March 2012).

- *Clinical Chemistry Trainee Council Webcasts and Pearls of Laboratory Medicine:*
  13. Browning T. Vitamin D. April 2012. Available from: http://www.traineecouncil.org/pearl (*Clinical Chemistry* Trainee Council, Pearls of Laboratory Medicine).

Tables

Tables are considered text. They should appear embedded as part of the submission directly following the reference section. Each table should appear on an individual page. Manuscripts will be returned to authors if the tables are uploaded as separate files.

Each table should be clearly labeled and numbered consecutively with Arabic numerals (Table 1, Table 2, etc.). The abbreviations used must be defined. As with the text, reporting of concentration units consists of analyte concentrations expressed in the text in the traditional mass unit (mg/dL, ng/ml, and so forth) followed by the SI unit in parentheses.

Tables should not be divided into parts. Separate parts (Table 1A, Table 1B, etc.) will be considered separate tables and will count toward your overall table/figure count.

Table captions should be limited to 60 words.

Each column of an individual table must have a heading and be clearly defined. Indicate explanatory footnotes with superscript lowercase italic letters in alphabetical order.

Tables will be converted into a standard format for publication that will not include color. Large or complex tables or tables that include graphic elements should be submitted as figures or as supplemental data for online publication only. The Editor may choose to make any table supplemental as he/she deems necessary.

Figure Captions

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