New insights into systemic amyloidosis: the importance of diagnosis of specific type

Maria M. Picken

Purpose of review

This review aims to summarize recent developments in the area of systemic amyloidoses with emphasis on pathologic diagnosis.

Recent findings

In recent years, management of amyloidosis has shifted from a purely supportive approach to quite diverse, radical and aggressive treatments. The central issue is the understanding that treatment of systemic amyloidoses depends on the molecular type of the amyloid protein. In the United States and the Western world, AL-amyloidosis is the most prevalent type of systemic amyloidosis, but hereditary amyloidoses are being diagnosed with increasing frequency; genetics also plays a role in a subset of familial AA amyloidoses. The biggest challenge is in the diagnosis of AL-type with confidence and in differentiation of AL and hereditary amyloidoses. While careful clinico-pathologic correlation is recommended for all patients with amyloidosis, it is, in itself, not a substitute for amyloid typing.

Summary

The diagnosis of the amyloid type ultimately depends on the examination of the amyloid protein within the deposits. The role of immunohistochemistry – the current standard of care in amyloid typing – is evolving with emergence of alternative biochemical methods. Amyloid, being essentially a protein disorder, presents an attractive venue for the application of proteomics methodologies, despite their inherent complexities.

Keywords

amyloid, diagnosis, hereditary amyloidosis, periodic fevers, systemic amyloidosis

Curr Opin Nephrol Hypertens 16:196–203. © 2007 Lippincott Williams & Wilkins.

Loyola University Medical Center, Maywood, Illinois, USA

Correspondence to Maria M. Picken MD, PhD, Pathology Bldg 110, Rm 2242, Loyola University Medical Center, 2160 S. First Avenue, Maywood, IL 60153, USA
Tel: +1 708 327 2607; fax: +1 708 327 2620; e-mail: mpicken@lumc.edu

Current Opinion in Nephrology and Hypertension 2007, 16:196–203

Abbreviations

AFib amyloid derived from fibrinogen A α chain
ATTR amyloid derived from tranthyretins
FMF familial Mediterranean fever

1062-4821

Introduction

Amyloid is a product of protein misfolding that results in extensive β-pleated sheet formation, which, in turn, is responsible for its affinity to the Congo red stain. Under polarized light, Congo red stained deposits exhibit an apple green birefringence, which is diagnostic of amyloid. By electron microscopy, amyloid is composed of fibrils 8–12 nm thick and of indefinite length [1]. Disease processes associated with amyloid formation – the amyloidoses – comprise a group of disorders of diverse cause: acquired or hereditary, neoplastic, infectious, degenerative or associated with ageing [1,2••]. Amyloid deposits can be derived from more than 25 different types of proteins and many more variants; additional types of amyloid protein and variants are likely to be discovered in the future. The current classification of amyloid is based upon the amyloid fibril protein type, also referred to as the precursor protein [1]. By convention, the amyloid type is designated A followed by an abbreviation derived from the name of the amyloid fibril precursor protein [1]. Amyloidosis can be cerebral or extracerebral, systemic, localized or localized and systemic [1,2••,3,4•–6•,7,8••,9••]. This review focuses on recent developments in the area of extracerebral systemic amyloidoses.

Systemic amyloidoses: overview

AL (also known as primary) amyloidosis is derived from the immunoglobulin light chain or, more frequently, a fragment thereof [2••,3,4•–6•,7,8••,9••]; rare forms of amyloid derived from truncated immunoglobulin γ or μ-heavy chain (termed AH) have also been reported [10–12]. AL-amyloidosis is associated with various B cell lymphoproliferative disorders encompassing the multiple myeloma-plasma cell dyscrasia spectrum and, on occasion, with malignant lymphomas and macroglobulinemia [13]. It is postulated that aminoacid substitutions in the light (or heavy) chain render them amyloidogenic. In the United States and the Western world, AL-amyloidosis is the most prevalent type of systemic amyloidosis [2••,3,4•–6•,7,8••]. Tumor-like, localized, AL-type deposits may be seen in some patients [14,15].

AA amyloidosis (formerly known as secondary) is more common in developing countries than AL-type [2••,4•,16] accounting for an estimated 45% of all systemic amyloidoses worldwide. Typically, AA amyloidosis is associated with ('secondary to') a chronic inflammatory process such as the chronic inflammatory arthritides (in the developed

world) and long-standing infections (in developing countries) [16,17]; an association with drug users and AIDS has also been reported [18,19]. Amyloid is derived from the serum protein precursor, SAA, which is typically upregulated in chronic inflammatory processes. Amyloid A protein is a product of incomplete proteolytic digestion of SAA [16].

Patients on long-term dialysis may develop amyloidosis derived from β_2-microglobulin ($A\beta_2$-m) [20,21].

Diversity of systemic amyloidoses: beyond AL and AA and the expanding role of genetics

Hereditary amyloidoses are being diagnosed with increasing frequency [22,23], yet recent data suggest that they are still underdiagnosed. Although these amyloidoses are autosomal dominant, the phenotype may vary significantly due to differences in gene penetration; de-novo mutations may also occur in some patients [24,25]. Hence, a family history may be missing and the clinical picture may mimic AL-type [24]. Thus, lack of awareness of the condition and the absence of a family history have had a major impact on substantial underdiagnosis. Although amyloid derived from tranthyretins (ATTR) was initially diagnosed in Portugal, Sweden and Japan, it is now recognized that ATTR is not limited to these geographic locations [22–24,26]. Presently, ATTR is the most common type of hereditary amyloidosis in the US [22]. Currently, over 100 mutations of transthyretin have been identified. Transthyretin circulates as a tetramer and is responsible for thyroid hormone transport and the binding of retinol. The presence of a mutant of the protein destabilizes the tetramer and leads to the release of monomers, which are believed to be amyloidogenic. Although ATTR is systemic, it is typically associated with polyneuropathy and cardiac involvement (more severe with some mutants) while renal involvement may be clinically silent. In some ATTR mutants, however, nephropathy may be present, leading to renal failure [27,28].

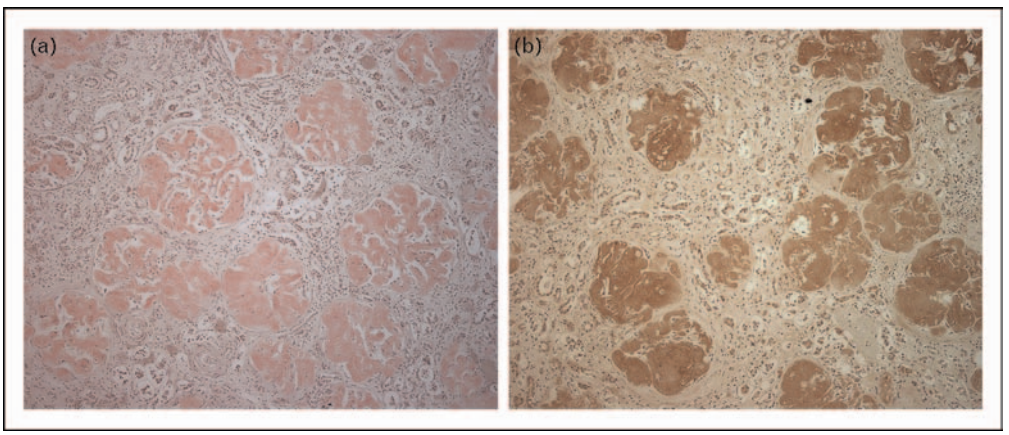
Interestingly, in elderly patients, the wild-type transthyretin may cause senile cardiac amyloidosis (SCA) [29–31]. Survival in SCA is significantly longer than in AL-type patients with cardiac involvement [31]. While SCA amyloid deposits may be systemic, extracardiac deposits (including renal) are usually clinically silent.

Amyloid derived from the fibrinogen A α chain (AFib) appears to be the most common form of hereditary amyloidosis in the UK and northern Europe [23,24,32]. Amyloid deposits in AFib are quite unique in that they appear to selectively target glomeruli and lead to their complete obliteration, with sparing of the extraglomerular compartments. Renal failure rapidly develops (Fig. 1).

In patients with systemic amyloidosis, presenting with renal, gastrointestinal or bleeding complications, amyloid derived from a mutant of lysozyme (ALys) should be considered [33,34]. In amyloidosis derived from apolipoprotein AI (AApo AI), deposits are typically extraglomerular, medullary and associated with renal failure rather than proteinuria (Fig. 2) [35]. Other types of amyloidosis affecting the kidney are listed in Table 1 [1,9••,22–24, 26–29,31–37,38•,39]. Although most of the hereditary amyloidoses can affect the kidney, the development of renal damage varies, ranging from slow to rapid progression to renal insufficiency; in some cases, deposits of amyloid may be clinically silent [32].

Genetics may play a role in other types of amyloidosis as well. An association between familial Mediterranean

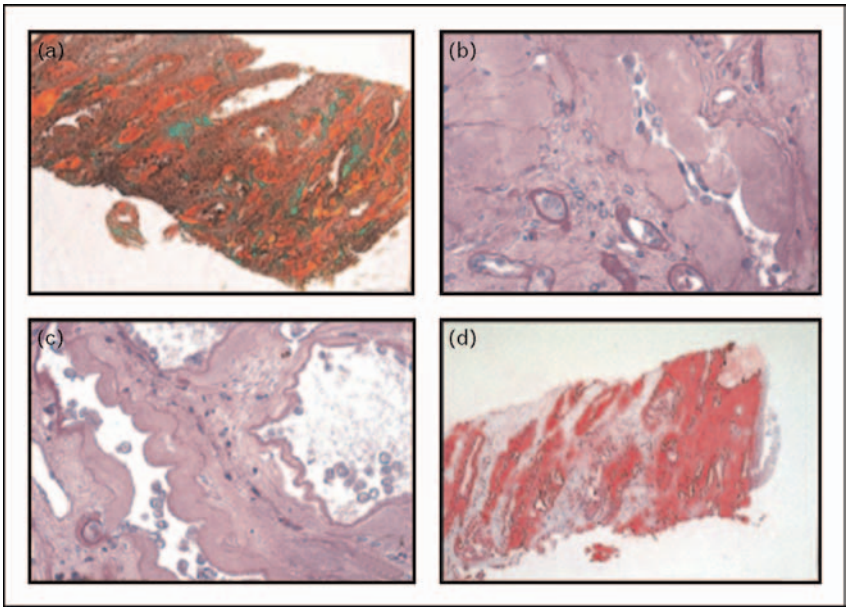
Figure 1 Kidney biopsy from a patient with amyloidosis derived from the Glu526Val variant of fibrinogen A α-chain



(a) Glomeruli are largely replaced by Congo red positive deposits. In contrast, the extraglomerular vessels and interstitium show little amyloid. These deposits were also birefringent when viewed under polarized light (not shown). Congo red stain, original mag. x100. (b) Immunohistochemical stain for fibrinogen is positive within the deposits of amyloid (original mag. x100). Reprinted with permission [24].

Figure 2 Kidney biopsy from a patient with amyloid derived from apolipoprotein AI

(a) Extensive peritubular and interstitial deposits of amyloid in the inner medulla. Congo red stained slide viewed under polarized light demonstrating apple green birefringence, original mag. x25. (b and c) Extensive peritubular and medullary deposits of amyloid. PAS stain, original mag. x40. 2D. Deposits of amyloid are immunoreactive with antibody against apolipoprotein AI. Original mag. x 40. Reprinted with permission [35].



fever (FMF) and AA amyloidosis has been recognized for a long time [38•,39–41]. Only recently, however, have the underlying genetic factors been understood [38•]. FMF is a prototype of the familial periodic fevers, also known as auto-inflammatory syndromes. Periodic fevers represent the recently expanding spectrum of chronic, recurrent inflammatory disorders associated with genetic defects in various enzymes involved in innate immunity (i.e. not associated with autoantibodies or antigen-specific T-cell activation). While some are recessive, others are autosomal dominant. Most patients have mutations in either pyrin, cryopyrin or the tumor-necrosis factor (TNF) receptor genes [38•,39–41]. Systemic AA amyloidosis develops in a subset of these patients with varying frequency, depending on the type of mutation [38•,39–41].

Table 1 Renal involvement in human systemic amyloidoses

Amyloid protein	Precursor	Syndrome
AL/AH	Immunoglobulin light/heavy chain	Multiple myeloma/plasma cell dyscrasia-associated, aka 'primary'
AA	Serum AA protein	Sporadic: secondary; Periodic fevers[a]: Familial Mediterranean fever, other
$A\beta_2M$	β_2-microglobulin	Dialysis-associated
ATTR	Transthyretin	Sporadic[b] Hereditary[c,d]
AFib	Fibrinogen A α-chain	Hereditary[c,e]
AApoAI	Apolipoprotein AI	Hereditary[c,f]
AApoAII	Apolipoprotein AII	Hereditary[c,g]
ALys	Lysozyme	Hereditary[c,h]
AGel	Gelsolin	Hereditary[c,i]
ACys	Cystatin C	Hereditary[c,j]

[a] Genetic defect in proteins involved in the inflammatory response but not the amyloid precursor protein per se [38•,39].
[b] Senile form derived from wild transthyretin with cardiomyopathy, renal involvement in medulla and vessels [29,31].
[c] Genetic defect involving the amyloid protein precursor [1,22].
[d] Polyneuropathy and cardiomyopathy, some mutants with severe renal involvement [26–28].
[e] Severe nephropathy with glomerular involvement [24].
[f] Hepatic involvement, cardiomyopathy, nephropathy with involvement of renal medulla, some mutants with neuropathy [35].
[g] Nephropathy [32,36].
[h] Gastrointestinal involvement, nephropathy [33,34].
[i] Cutis laxa, cranial neuropathy, renal failure in homozygotes [37].
[j] Clinically silent [23]. Adapted with permission [9••].

FMF has been associated with the Mediterranean basin, while several other periodic fevers were detected in northern Europe. Recently, however, additional FMF and other periodic fever patients have been identified throughout Europe and the US [38•,39–43]. Periodic fevers are likely to have been underdiagnosed. The availability of genetic testing, and increased awareness, have led to an increased recognition of these disorders. Recently, genetic defects in genes involved in innate immunity have been demonstrated in several patients who developed AA amyloidosis, seemingly without evidence of an underlying chronic inflammation [17].

Advances in treatment of systemic amyloidoses

In recent years, management of amyloidosis has undergone a formidable evolution: it has shifted from a purely supportive approach to quite diverse, radical and aggressive treatments [44,45••]. The central issue is the understanding that treatment of systemic amyloidoses depends on the molecular type of the amyloid protein.

For AL amyloidosis, various chemotherapy regimens have evolved over the last decade. A durable response can be achieved in AL patients using high-dose melphalan with autologous peripheral stem-cell rescue [3,4•,5•,7,8••,44,45••,46,47]. Other regimens (tandem transplants) and agents (thalidomide and its derivatives) are also being tested [48–50]. These regimens have been shown to control the underlying plasma cell dyscrasia leading to a reduction in the level of free serum light chains; a gradual improvement in the function of affected organs also occurs [51–53]. Treatment outcomes are better with early therapy and in patients with preserved renal function [6•,44]. Owing to significant treatment-related mortality and morbidity, patient selection for treatment has to be rigorous. In the past, patients over 65 years old and patients with cardiac amyloid were considered unsuitable for such a high risk therapy. Recently, however, patients over 65 years old were also successfully treated with a risk-adapted therapy [54]. Moreover, for patients with cardiac AL amyloidosis, sequential heart and autologous stem cell transplantation have been tried [55]. Living donor kidney and autologous stem cell transplantation for AL amyloidosis with predominant renal involvement was recently reported [56]. A consensus definition of organ involvement and treatment response in AL-type was also recently published [57].

Since the early 1990s, liver transplantation has been offered to patients with ATTR; the rationale being that, since the liver was the main source of abnormal protein, its surgical replacement by an organ that produces normal transthyretin, should cure the condition [58]. Given the relatively high morbidity associated with liver transplantation, surgery was initially offered only to patients with established disease. It is now, however, widely recognized that better results can be achieved in patients who are transplanted at an early stage of amyloidosis [59,60]. In patients with severe cardiac or renal involvement, liver transplantation was combined with heart or kidney transplantation. Interestingly, heavy amyloid deposits in the kidneys, especially in the glomeruli, may portend a poor outcome for liver transplantation [61]. Thus, kidney biopsy has been proposed as an outcome predictor for liver transplantation. Hepatorenal transplantation has also been successfully offered to patients with hereditary amyloidosis derived from apolipoprotein AI, apolipoprotein AII and fibrinogen, with excellent results [36,62,63].

Nonsurgical therapies are also being developed for ATTR [64–66]. Several small molecules have been identified that can stabilize the transthyretin tetramer in the presence of mutant subunits and thus prevent the release of pathogenic monomers. Currently, one such molecule – diflunisal – is in clinical trial [65].

In AA amyloidosis, control of the underlying infection is currently the standard of care. A new therapy, however, is also being tested in a clinical trial. A small molecule (eprodisate disodium, Kiacta TM, formerly Fibrillex TM) has been shown, *in vivo*, to inhibit fibrillogenesis [67].

In FMF, colchicine has been effectively used for decades to control the inflammatory response and prevent AA amyloidosis [41]. In other periodic fevers, biologic response modifiers, such as inhibitors of TNF and IL-1β, are being tested [68].

Localized amyloid deposits, which may be encountered in various organs, do not warrant systemic treatment [14,15].

Diagnosis of amyloidosis: detection and typing

Congo red stain continues to be the gold standard in the diagnosis of amyloid (reviewed [2••,9••]). Clearly, more sensitive and easier screening methods for the diagnosis of amyloid are needed. In this connection, preliminary studies [69] reporting the application of an amyloid chip to generic detection of amyloid in tissue biopsy samples are interesting.

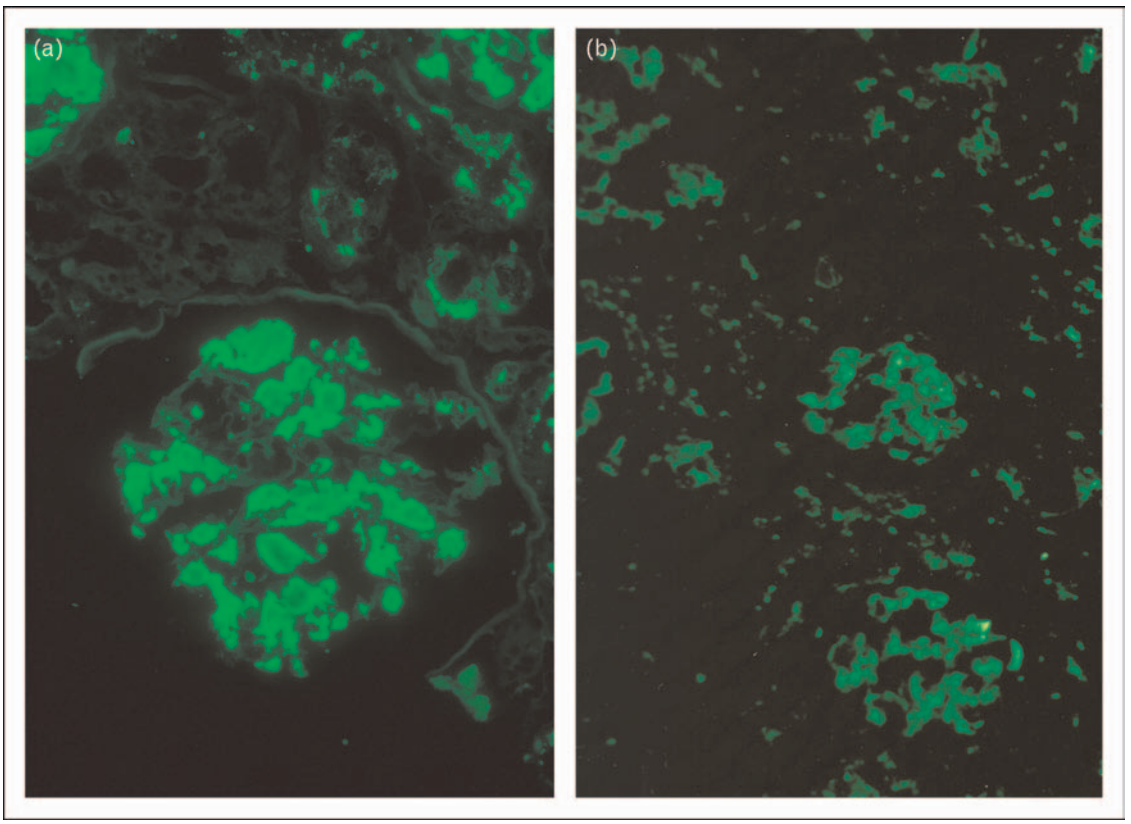
It must be stressed that potassium permanganate stain currently has no role in amyloid typing. Immunohistochemical diagnosis of AA-type is relatively reliable; the biggest challenge is in the diagnosis and differentiation of AL and hereditary amyloidoses [3,5•,44].

In AL-type, due to light chain truncation, amyloid fibril proteins may be composed of a variable region that is largely devoid of the constant region fragment. Such proteins may be nonreactive with commercial antibodies, which are typically raised against the C region. Thus, it may be anticipated that not all AL amyloid deposits will be reactive with commercial antibodies for κ or λ light chains [9••].

Review of recently published series [9••,24,70–72] shows quite a wide range of success in immunohistochemical amyloid typing, ranging from 38 to 87%. Interestingly, other antibody-based techniques, such as immunoelectron microscopy and Western blotting, have been shown to yield better results than immunohistochemistry [73,74,75•]. Thus, antigenic preservation appears to play some role. The second issue is that of 'contamination' by serum proteins, which may create a background stain.

Interestingly, reports from nonrenal referral centers show a lower rate of success. While this may be, in part, a consequence of more stringent diagnostic criteria, technical issues should also be considered. Thus, amyloid typing in frozen tissue, typically used in renal pathology, yield much better results, with success rates between 65 and 87%

Figure 3 Kidney biopsy from a patient with AL amyloidosis, frozen section, direct immunofluorescence stain



(a) Bright immunofluorescence stain for λ light chain outlines deposits of amyloid within glomeruli and in the interstitium, original mag. x200. Stain for κ light chain was negative (not shown). (b) Amyloid deposits show strong positivity for amyloid P component. Amyloid P component is present in all types of amyloid deposits regardless of their chemical composition. Original mag. x100. Both stains, for λ light chain and amyloid P component, correspond to Congo red positive areas (not shown). Reprinted with permission [9••].

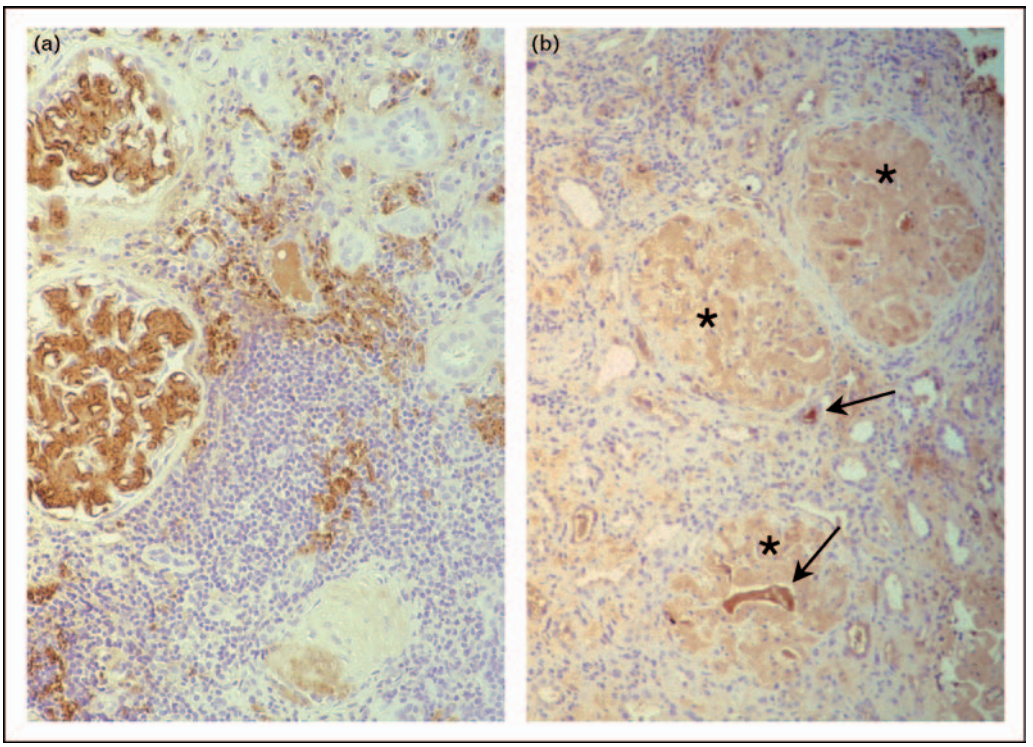
[9••,71,72]. In the author's own experience, background 'contamination' with serum proteins is much less bothersome in frozen sections (Figs 3 and 4). Therefore, immunofluorescence is the technique of choice for typing amyloid derived from immunoglobulin light and heavy chains and, indeed, amyloid in general [2••,9••,72].

In order to compensate for background stain, the use of a panel of antibodies is recommended in that it provides an opportunity to identify the strongest antibody reaction. The choice of antibodies in a panel should be a reasonable compromise between the ever-increasing diversity of amyloidoses, their clinical relevance in terms of available treatments, the volume of testing and the availability of adequate controls [2••,9••]. It is also clear, however, that even for a first attempt at amyloid typing, antibodies against the most common hereditary amyloidoses – ATTR and AFib – should be included. In fact, ATTR is the second most common type of amyloid diagnosed in myocardial and peripheral nerve biopsies.

Recently, the importance of discrimination between AL and hereditary amyloidoses has been recognized. Two studies [24,76•] have demonstrated that hereditary amyloidosis may be misdiagnosed as AL in some patients. In one series [24] of 350 patients with a presumptive diagnosis of AL amyloidosis and the absence of a family history, 10% of patients were shown to have familial amyloidosis. Twenty-four percent of these patients also had a low-grade monoclonal gammopathy. In a study by Comenzo *et al.* [76•], 6% of screened patients and 2% of asymptomatic patients had both a monoclonal gammopathy and a mutation in an amyloidogenic protein. Thus, it is currently recognized that the exclusion of other types of amyloid is an important part of the work-up in patients with amyloidoses [24,76•].

It has also been shown that the presence of a mutation within the sequence of an amyloidogenic protein does not necessarily indicate that the amyloid in the patient is derived from that mutated protein. To this end, patients with proven AL amyloidosis, who also had mutations in one of the amyloidogenic proteins, were recently reported [77]; examples of AL, diagnosed in a clinical setting suggestive of AA amyloidosis, have also been demonstrated [78–80]. Thus, it is imperative that the criteria for amyloid-type diagnoses be stringent.

Figure 4 AL-amyloidosis, paraffin sections, immunoperoxidase stain



(a) Strong stain for λ light chain in the glomeruli and interstitium corresponding to deposits of amyloid, same specimen as Fig. 3a. Original mag. x150. (b) In contrast, stain for κ light chain shows only focal positivity corresponding to serum proteins (arrow) and only a 'blush' stain in areas corresponding to amyloid (asterisks), same specimen as (a). Original mag x150. Reprinted with permission [9••].

Biochemical typing of amyloid protein extracted from formalin-fixed, paraffin-embedded specimens has been tried for several years [81]. Most recently, the application of proteomics techniques to amyloid typing has been reported [82•,83]. This requires lesser amounts of material for study, but at the expense of complexity of preparation. While such studies are feasible in paraffin-embedded biopsies, fresh, unfixed tissue is preferable. Interestingly, adipose tissue may be a reasonable source of such samples [84••].

Although these sophisticated (and labor intensive) techniques are currently available only in highly specialized research laboratories, and have not yet been validated in large numbers of samples in multiple centers, their development is certainly a welcome advancement. While it is appealing to see the future of amyloid diagnosis in proteomics, the question arises as to whether amyloid proteomics is ready for prime time now [85•,86•].

Conclusion

The clinical relevance of diagnosis of amyloidosis cannot be overestimated. As the treatment options expand, there is increasing need for early diagnosis and accurate amyloid typing. Currently, immunohistochemistry is the standard technique used for typing of amyloid. It is critical that amyloid typing is done properly. In inconclusive cases, additional studies, best performed in specialized centers, are needed.

Since amyloid is essentially a protein disorder, proteomics methodologies are emerging as applicable diagnostic tools, despite their inherent complexities.

Acknowledgements

Supported in part by a research grant from the Finkl-Amyloidosis Foundation. Thanks to Roger N. Picken PhD for a critical review of the manuscript.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 275–276).

1 Westermark P, Benson MD, Buxbaum JN, *et al.* Nomenclature Committee of the International Society of Amyloidosis. Amyloid: toward terminology clarification. Report from the Nomenclature Committee of the International Society of Amyloidosis. Amyloid 2005; 12:1–4.

2 •• Herrera GA, Picken MM. Renal Diseases associated with plasma cell dyscrasias, amyloidoses, Waldenstrom macroglobulinemia and cryoglobulinemic nephropathies. In: Jennette JC, Olson JL, Schwartz MM, Silva FG, editors. Heptinstall's Pathology of the Kidney, 6th ed. Philadelphia: Lippincott Williams and Wilkins; 2006 pp. 853–910.
This is an updated overview of amyloidoses and comprehensive review of renal pathology associated with plasma cell dyscrasia and amyloidoses in general.

3 Obici L, Perfetti V, Palladini G, *et al.* Clinical aspects of systemic amyloidosis. Bioch Biophys Acta 2005; 1253:11–22.

4 • Lachmann HJ, Hawkins PN. Systemic amyloidosis. Curr Opinion Pharmacol 2006; 6:1–7.
This is a recent overview of systemic amyloidoses emphasizing new therapeutic strategies.

5 • Comenzo R. Amyloidosis. Curr Treat Options Oncol 2006; 7:225–236.
This provides an update on systemic amyloidoses with emphasis on the clinical picture and treatments.

6 • Dember LM. Amyloidosis-associated kidney disease. J Am Soc Nephrol 2006; 17:3458–3471.
This recent review focuses on renal amyloidoses.

7 Gertz MA, Lacy MQ, Dispenzieri A, Hayman SR. Amyloidosis: diagnosis and management. Clin Lymphoma Myeloma 2005; 6:208–219.

8 •• Sanchorawala V, Blanchard E, Seldin DC, *et al.* AL amyloidosis associated with B-cell lymphoproliferative disorders: frequency and treatment outcomes. Am J Hematol 2006; 81:692–695.
The authors report the largest series and longest follow-up of high-dose chemotherapy and autologous stem cell rescue in AL amyloidosis.

9 •• Picken MM. Immunoglobulin light and heavy chain amyloidosis AL/AH: renal pathology and differential diagnosis. Contrib Nephrol 2007; 153:135–155.
This is a comprehensive updated review of pathology and differential diagnosis of amyloidoses with emphasis on renal amyloidoses. Current diagnostic standards are reviewed, including interpretation of Congo red stain and alternative methods as well as proposed recommendations for amyloid typing.

10 Gono T, Yazaki M, Fushimi T, *et al.* AH amyloidosis associated with lymphoplasmacytic lymphoma secreting a monoclonal gamma heavy chain carrying an unusual truncated D segment. Am J Kid Dis 2006; 47:908–914.

11 Nasr SH, Colvin R, Markowitz GS. IgG1 lambda light and heavy chain renal amyloidosis. Kidney Int 2006; 70:7.

12 Komatsuda A, Maki N, Wakui H, *et al.* Development of systemic lambda-light chain amyloidosis in a patient with gamma-heavy chain deposition disease during long-term follow-up. Nephrol Dial Transplant 2005; 20:434–437.

13 Gertz MA, Merlini G, Treon SP. Amyloidosis and Waldenstrom's macroglobulinemia. Hematology Am Soc Hematol Educ Program 2004; 257–282.

14 Paccalin M, Hachulla E, Cazalet C, *et al.* Localized amyloidosis: a survey of 35 French cases. Amyloid 2005; 12:239–245.

15 Biewend M, Menke DM, Calamia KT. The spectrum of localized amyloidosis: a case series of 20 patients and review of the literature. Amyloid 2006; 13:135–142.

16 Rocken C, Shakespeare A. Pathology, diagnosis and pathogenesis of AA amyloidosis. Virchows Arch 2002; 440:111–122.

17 Lachmann HJ, Goodman HJB, Gallimore J, *et al.* Characteristics and clinical outcome of 340 patients with systemic AA amyloidosis. In: Grateau G, Kyle RA, Skinner M, editors. Amyloid and amyloidosis. Boca Raton, FL: CRC Press; 2005. pp. 173–175.

18 Connolly JO, Gillmore JD, Lachmann HJ, *et al.* Renal amyloidosis in intravenous drug users. QJM 2006; 99:737–742.

19 Atta MG, Choi MJ, Longenecker JC, *et al.* Nephrotic range proteinuria and CD4 count as noninvasive indicators of HIV-associated nephropathy. Am J Med 2005; 118:1288.

20 Saito A, Gejyo F. Current clinical aspects of dialysis-related amyloidosis in chronic dialysis patients. Ther Apher Dial 2006; 10:316–320.

21 Dember LM, Jaber BL. Dialysis-related amyloidosis: late finding or hidden epidemic? Semin Dial 2006; 19:105–109.

22 Connors LH, Prokayeva T, Akar H, *et al.* Familial amyloidosis: recent novel and rare mutations in a clinical population. In: Grateau G, Kyle RA, Skinner M, editors. Amyloid and Amyloidosis. Boca Raton, FL: CRC Press; 2005. pp. 360–362.

23 Hawkins PN. Hereditary systemic amyloidoses with renal involvement. J Nephrol 2003; 16:443–448.

24 Lachmann HJ, Booth DR, Booth SE, *et al.* Misdiagnosis of hereditary amyloidosis as AL (primary) amyloidosis. N Engl J Med 2002; 346:1786–1791.

25 Kang HG, Bybee A, Ha IS, *et al.* Hereditary amyloidosis in early childhood associated with a novel insertion-deletion (indel) in the fibrinogen A alpha chain gene. Kidney Int 2005; 68:1994–1998.

26 Ando Y, Nakamura M, Araki S. Transthyretin-related familial amyloidotic polyneuropathy. Arch Neurol 2005; 62:1057–1062.

27 Lobato L, Beirao I, Silva M, *et al.* End-stage disease and dialysis in hereditary amyloidosis TTR V30M: presentation, survival and prognostic factors. Amyloid 2004; 11:27–37.

28 Haagsma EB, Hawkins PN, Benson MD, *et al.* Familial amyloidotic polyneuropathy with severe renal involvement in association with transthyretin Gly47Glu in Dutch, British and American-Finnish families. Amyloid 2004; 11:44–49.

29 Westermark P, Bergstrom J, Solomon A, *et al.* Transthyretin-derived senile systemic amyloidosis: clinicopathologic and structural considerations. Amyloid 2003; 1:48–54.

30 Picken MM, Leya F, Picken RN. Wild type transthyretin-derived senile systemic amyloidosis with limited proteolytic digestion of the monomer: clinico-pathologic studies with DNA and amyloid protein characterization. In: Grateau G, Kyle RA, Skinner M, editors. Amyloid and amyloidosis. Boca Raton, FL: CRC Press; 2005. pp. 466–467.

31 Ng B, Connors LH, Davidoff R, *et al.* Senile systemic amyloidosis presenting with heart failure. Arch Intern Med 2005; 165:1425–1429.

32 Benson MD. Ostertag revisited: the inherited systemic amyloidoses without neuropathy. Amyloid 2005; 12:75–87.

33 Granel B, Valleix S, Serratrice J, *et al.* Lysozyme amyloidosis: report of 4 cases and a review of the literature. Medicine 2006; 85:66–73.

34 Loss M, Ng WS, Karim RZ, *et al.* Hereditary lysozyme amyloidosis: spontaneous hepatic rupture (15 years apart) in mother and daughter, role of emergency transplantation. Liver Transpl 2006; 12:1152–1155.

35 Gregorini G, Izzi C, Obici L, *et al.* Renal apolipoprotein A-I amyloidosis: a rare and usually ignored cause of hereditary tubulointerstitial nephritis. J Am Soc Nephrol 2005; 16:3680–3686.

36 Magy N, Liepnieks JJ, Yazaki M, *et al.* Renal transplantation for apolipoprotein AII amyloidosis. Amyloid 2003; 10:224–228.

37 Ardalan MR, Shoja MM, Kiuru-Enari S. Amyloidosis-related nephritic syndrome due to a G654A gelsolin mutation: the first report from the Middle East. Nephrol Dial Transplant 2006; Sep 23. [Epub ahead of print]

38 • Brydges S, Kastner DL. The systemic autoinflammatory diseases: inborn errors of the innate immune system. Curr Top Microbiol Immunol 2006; 305:586–599.
This is a comprehensive review of recent advances in the understanding of inflammation in general and auto-inflammatory diseases that carry a high risk of AA amyloidosis in particular.

39 Grateau G, Jeru I, Rouaghe S, *et al.* Amyloidosis and auto-inflammatory syndromes. Curr Drug targets: inflammation and allergy 2005; 4:57–65.

40 Padeh S. Periodic fever syndromes. Pediatr Clin N Am 2005; 52:577–609.

41 Ben-Chetrit E, Bergmann S, Sood R. Mechanism of the anti-inflammatory effect of colchicines in rheumatic diseases: a possible new outlook through microarray analysis. Rheumatology (Oxford) 2006; 45:274–282.

42 Dinc A, Erdem H, Rowczenio D, *et al.* Autosomal dominant periodic fever with AA amyloidosis: tumor necrosis factor receptor-associated periodic syndrome (TRAPS) in a Turkish family. J Nephrol 2005; 18:626–629.

43 Lachmann HJ, Goodman HJ, Andrews PA, *et al.* AA amyloidosis complication hyperimmunoglobulinemia D with periodic fever syndrome: a report of two cases. Arthritis Rheum 2006; 54:2010–2014.

44 Dember LM. Emerging treatment approaches for systemic amyloidoses. Kidney Int 2005; 68:1377–1390.

45 •• Comenzo RL. Current and emerging views and treatments of systemic immunoglobulin light-chain (AL) amyloidosis. Contrib Nephrol 2007; 153: 195–210.
This is an excellent recent review of the subject.

46 Goodman HJ, Gillmore JD, Lachmann HJ, *et al.* Outcome of autologous stem cell transplantation for AL amyloidosis in the UK. Br J Haematol 2006; 134:417–425.

47 Schonland SO, Lockhorts H, Buzyn A, *et al.* Allogeneic and syngeneic hematopoetic cell transplantation in patients with amyloid light chain amyloidosis: a report from the European Group for Blood and Bone Marrow Transplantation. Blood 2006; 107:2578–2584.

48 Wechelakar AD, Goodman HJ, Lachmann HJ, *et al.* Safety and efficacy of risk adapted cyclophosphamide, thalidomide and dexamethasone in systemic AL amyloidosis. Blood 2006; Sep 21. [Epub ahead of print]

49 Kristen AV, Myer FJ, Perz JB, *et al.* Risk stratification in cardiac amyloidosis: novel approaches. Transplantation 2005; 80:S151–S155.

50 Sanchorawala V, Wright DG, Rosenzweig M, *et al.* Lenalidomide and dexamethasone in the treatment of AL amyloidosis: results of a phase II trial. Blood 2006; Sep 7. [Epub ahead of print]

51 Sanchorawala V, Seldin DC, Magnani B, *et al.* Serum free light-chain responses after high-dose intravenous melphalan and autologous stem cell transplantation for AL (primary) amyloidosis. Bone Marrow Transplant 2005; 36:597–600.

52 Palladini G, Lavatelli F, Russo P, *et al.* Circulating amyloidogenic free light chains and serum N-terminal natriuretic peptide B decrease simultaneously in association with improvement of survival in AL. Blood 2006; 107:3854–3858.

53 Dispenzieri A, Lacy MQ, Katzmann JA, *et al.* Absolute values of immunoglobulin free light chains are prognostic in patients with primary systemic amyloidosis undergoing peripheral blood stem cell transplant. Blood 2006; 107:3378–3383.

54 Seldin DC, Anderson JJ, Skinner M, *et al.* Successful treatment of AL amyloidosis with high-dose melphalan and autologous stem cell transplantation in patients over age 65. Blood 2006; 108:3945–3947.

55 Gillmore JD, Goodman HJ, Lachmann HJ, *et al.* Sequential heart and autologous stem cell transplantation for systemic AL amyloidosis. Blood 2006; 107:1227–1229.

56 Leung N, Griffin MD, Dispenzieri A, *et al.* Living donor kidney and autologous stem cell transplantation for primary systemic amyloidosis (AL) with predominant renal involvement. Am J Transplant 2005; 5:1660–1670.

57 Gertz MA, Comenzo R, Falk RH, *et al.* Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis. Am J Hematol 2005; 79:319–328.

58 Wilczek HE, Larsson M, Ericzon BG. Report from the Familial Amyloidotic Polyneuropathy World Transplant Registry (FAPWTR) and the Domino Liver Transplant Registry (DLTR) [abstract]. Transplantation 2006; 82 (1 Suppl 2): 152A.

59 Suhr OB, Friman S, Ericzon BG. Early liver transplantation improves familial amyloidotic polyneuropathy patients' survival. Amyloid 2005; 12:233–238.

60 Nowak G, Suhr OB, Wikstrom L, *et al.* The long-term impact of liver transplantation on kidney function in familial amyloidotic polyneuropathy patients. Transpl Int 2005; 18:111–115.

61 Oguchi K, Takei Y, Ikeda S. Value of renal biopsy in the prognosis of liver transplantation in familial amyloid polyneuropathy ATTR Val30Met patients. Amyloid 2006; 13:99–107.

62 Mousson C, Heyd B, Justrabo E, *et al.* Successful hepatorenal transplantation in hereditary amyloidosis caused by frame-shift mutation in fibrinogen A alpha-chain gene. Am J Transplant 2006; 6:632–635.

63 Gillmore JD, Stangou AJ, Lachmann HJ, *et al.* Organ transplantation in hereditary apolipoprotein AI amyloidosis. Am J Transplant 2006; 6:2342–2347.

64 Sekijima Y, Dendle MA, Kelly JW. Orally administered diflunisal stabilizes transthyretin against dissociation required for amyloidogenesis. Amyloid 2006; 13:236–249.

65 Berk J, Bisbee J, Kelly J, *et al.* Clinical trial for TTR amyloidosis using diflunisal. Amyloid 2006; 13 (Suppl 1):11A.

66 Benson MD, Kluve-Beckerman B, Zeldenrust SR, *et al.* Targeted suppression of an amyloidogenic transthyretin with antisense oligonucleotides. Muscle Nerve 2006; 33:609–618.

67 Dember L, Lachmann H, Obici L, *et al.* Eprodisate (NC-503) treatment of AA amyloidosis: open label follow-up of a randomized controlled trial. J Am Soc Nephrol 2006; 17:566A.

68 Hawkins PN, Lachmann HJ, Aganna E, *et al.* Spectrum of clinical features in Muckle-Wells syndrome and response to Anakinra. Arthritis Rheum 2004; 50:607–612.

69 Lomas L, Hazenberg BPC, Picken MM. Tissue detection of amyloid by an amyloid 'chip'? Amyloid 2006; 13 (Suppl 1):43A.

70 Kebbel A, Rocken C. Immunohistochemical classification of amyloid in surgical pathology revisited. Am J Surg Pathol 2006; 30:673–683.

71 Novak L, Cook WJ, Herrera GA, *et al.* AL-amyloidosis is underdiagnosed in renal biopsies. Nephrol Dial Transplant 2004; 19:3050–3053.

72 Picken MM. Amyloid typing in pathology: experience of a single institution. Amyloid 2006; 13 (Suppl 1):53A.

73 Veeramachaneni R, Gu X, Herrera GA. Atypical amyloidosis: diagnostic challenges and the role of immunoelectron microscopy in diagnosis. Ultrastruct Pathol 2004; 28:75–82.

74 Anesi E, Palladini G, Perfetti V, *et al.* Therapeutic advances demand accurate typing of amyloid deposits. Am J Med 2001; 111:243–244.

75 • Westermark P, Davey E, Lindbom K, Enqvist S. Subcutaneous fat tissue for diagnosis and studies of systemic amyloidosis. Acta histochem 2006; 108:209–213.

The authors describe application of Western blot to amyloid typing.

76 • Comenzo RL, Zhou P, Fleisher M, *et al.* Seeking confidence in the diagnosis of systemic AL (Ig light chain) amyloidosis: patients can have both monoclonal gammopathies and hereditary amyloid proteins. Blood 2006; 107:3489–3491.

This important report extends a prior study by Lachmann *et al.* [24] addressing the issue of AL mimickers and misdiagnosis. In this study, 6% of screened patients and 2% of asymptomatic patients had both a monoclonal gammopathy and a mutation in an amyloidogenic protein.

77 Landau H, Comenzo RL, Zhou P, *et al.* AL amyloidosis in a patient with a T60A TTR mutation. Amyloid 2006; 13 (Suppl 1):40A.

78 Oshima K, Kracker D, Picken MM. AL in the clinical setting of AA: the importance of amyloid typing. Amyloid 2006; 13 (Suppl 1):51A.

79 Pettersson T, Antilla P, Tornroth T. Renal glomerular AL and vascular AA amyloidosis in a patient with ankylosing spondylitis. In: Grateau G, Kyle RA, Skinner M, editors. Amyloid and amyloidosis. Boca Raton, FL: CRC Press; 2005. pp. 284–285.

80 Kracker D, Litbarg N, Picken MM. Amyloidosis in ankylosing spondylitis: unexpected findings underscoring the importance of typing amyloid deposits. Amyloid 2006; 13 (Suppl 1):38A.

81 Kaplan B, Martin BM, Livneh A, *et al.* Biochemical subtyping of amyloid in formalin-fixed tissue samples confirms and supplements immunohistologic data. Am J Clin Pathol 2004; 121:794–800.

82 • Murphy CL, Wang S, Williams T, *et al.* Characterization of systemic amyloid deposits by mass spectrometry. Methods Enzymol 2006; 412: 48–62.

This provides an extended protocol for application of proteomics to amyloid typing.

83 Lavatelli F, Perlman DH, McComb ME, *et al.* A proteomic approach to the study of systemic amyloidoses. Amyloid 2006; 13 (Suppl 1):13A.

84 •• van Gameren II, Hazenberg BP, Bijzet J, van Rijswik MH. Diagnostic accuracy of subcutaneous abdominal fat tissue aspiration for detecting systemic amyloidosis and its utility in clinical practice. Arthritis Rheum 2006; 54: 2015–2021.

The authors have published a number of papers on technical issues involved in obtaining an adequate sample of fat for diagnosis and quantitation of the amount of amyloid in response to treatment, using a specially devised scoring system.

85 • Solomon A, Murphy CL, Westermark P. Misclassification of amyloidosis is unwarranted. Blood 2006; 108:776.

Important comments are made pertaining to applicability of proteomics to amyloid typing stressing the pro-application arguments.

86 • Comenzo RL. Constraints on the pursuit of diagnostic confidence. Blood 2006; 108:776–777.

Important comments are made pertaining to applicability of proteomics to amyloid typing emphasizing current constraints.