

Amyloidosis and Auto-Inflammatory Syndromes

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Abstract: Amyloidosis remains currently a severe potential complication of many chronic inflammatory disorders. It is not exactly known why some patients develop a progressive amyloidosis, whereas others do not although latent deposits may be present. A permanent acute phase response, ideally evaluated with serial measurement of serum protein SAA, the precursor of the AA protein deposited in tissues, seems to be a prerequisite to the development of inflammatory (AA) amyloidosis. Genetic factors have however been recently emphasized. Among persistent or emerging causes of AA amyloidosis, hereditary periodic fever syndromes also known as auto-inflammatory syndromes are a group of diseases characterised by intermittent bouts of clinical inflammation with focal organ involvement mainly: abdomen, musculoskeletal system and skin. The most frequent is familial Mediterranean fever which affects patients of Mediterranean descent all over the world. Three other types have been recently clinically as well as genetically characterised. A thorough diagnosis is warranted, as clinical and therapeutic management is specific for each of these diseases.

THE GENERAL CONCEPT OF AMYLOIDOSIS

Amyloid was first described in the nineteenth century as an extracellular material found in tissues of patients affected with chronic infectious diseases [1]. Amyloid has then been observed in various clinical settings leading to a first clinical classification of amyloidoses, which are

the diseases associated with amyloid deposits. Four main entities were thus recognized: primary amyloidosis (i.e. amyloidosis without any detectable infectious cause), secondary amyloidosis (to an infectious disease), hereditary and localized types. A dramatic change in our knowledge of amyloid occurred in 1971, when Glenner showed that the main protein constituent of amyloid in primary amyloidosis was derived from a light

Table 1. Amyloid Proteins and Their Precursors

| Amyloid protein | Precursor | Diffusion | Disease or affected tissues |
|--------------------|---|-----------|--|
| AL | Ig light chain (κ, λ) | L | (primary) isolated or myeloma-associated |
| AH | Ig heavy chain | G, L | isolated |
| AA | (apo) SAA | G, L | (reactive) infection, chronic inflammatory disease, tumor, auto-inflammatory syndromes |
| ATTR | mutated transthyretin | G | hereditary |
| | normal transthyretin | G | senile |
| A β 2M | β 2-microglobulin | G | chronic renal failure |
| AApoAI | apolipoproteinAI | G | hereditary |
| | | L | aortic |
| AApoAII | apolipoproteinAII | G | hereditary |
| AGel | gelsolin | G | hereditary |
| ALys | lysozyme | G | hereditary |
| AFib | fibrinogen | G | hereditary |
| ACys | cystatin C | L | cerebral hemorrhage Icelandic type |
| A β | A β precursor protein: (A β PP) | L | Alzheimer's disease, Down syndrome, amyloid angiopathy hereditary or sporadic |
| APrP ^{SC} | prion protein | L | spongiform encephalopathy |
| ACal | (pro)calcitonin | L | medullary thyroid carcinoma |
| AANF | atrial natriuretic factor | L | atrial amyloid |
| AIAPP | amylin | L | Langerhans islets in type 2 diabetes, insulinoma |
| AIns | insulin | L | iatrogenic |
| APro | prolactin | L | prolactinoma, pituitary gland |
| AKep | keratopithelin | | corneal |
| ABri* | | | british hereditary dementia |
| AMed | lactadherin | | aortic (media) |

G: generalised; L: localised
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chain of immunoglobulin [2]. This can be considered as the beginning of the biochemical elucidation of the amyloid phenomenon, and led to the current classification and nomenclature of amyloidosis (Table 1) [3]. This classification is indeed based on the biochemical nature of the amyloid protein that is deposited in tissues. More than 20 proteins, which do not share any constant similarities based on primary structure or function, have thus been isolated in humans from various types of deposits. Amyloidosis is thus a common final pathway for an abnormal metabolism of many proteins *in vivo*, characterised by the acquisition by the amyloid protein of a prominent β -sheeted structure, followed by a unique self-aggregation. This last step requires an interaction with the extracellular matrix (EM). Amyloidosis has thus shifted from the status of storage disease to that of a protein folding disorder, with potential consequences for the therapeutic of this disorder, which remains so far, limited [4].

We will in this chapter describe the current status of the epidemiology of multisystemic amyloidoses, the approach of a thorough diagnosis of the clinical and biochemical nature of amyloidosis, and then focus on amyloidosis as a complication of auto-inflammatory syndromes.

EPIDEMIOLOGY OF MULTISYSTEMIC AMYLOIDOSIS

In a general way, epidemiological data concerning multisystemic amyloidoses are scarce. Most studies do not distinguish AA from immunoglobulin AL amyloidosis and many are biased as the diagnosis of amyloidosis was obtained at autopsy. In the United States of America, an estimation of the annual incidence of light chain amyloidosis (AL), based on mortality and population data ranges from 4.5 to 8.9/million [5]. In the Netherlands, the annual incidence of all generalised amyloidoses, would be 13.3/million [6]. An approach to the incidence of AA amyloidosis is thus to determine the AL/AA ratio. In the Olmsted county (USA) the ratio AL/AA is 17/1, whereas in The Netherlands it would be 1/2. However these data are certainly biased by the recruitment of these tertiary care units and this discrepancy is to be elucidated. A retrospective study in a general university hospital in France suggests a 3/1 AL/AA ratio [7]. These results do not indicate with certitude either that AA amyloidosis has disappeared in the United States or that the incidence of AL amyloidosis is increasing. The "true" incidence and prevalence of the main types of multisystemic amyloidoses (AL, AA and common hereditary varieties) remains to be known by prospective studies in Europe as well as in the

United States. Some data are available on hereditary forms of multisystemic amyloidosis. A specific V122I transthyretin variant has been found to be present at the heterozygous state in 3% of Black Americans [8].

Table 2 indicates diseases associated with AA amyloidosis in 5 series of patients over the last forty years: inflammatory diseases have replaced infectious diseases in the Western world [9-13]. Rheumatoid arthritis is currently the most frequent cause of AA amyloidosis, followed by ankylosing spondylarthritis, inflammatory bowel diseases and familial Mediterranean fever. A population-based study from Finland of 1666 patients with rheumatoid arthritis who died, indicates a prevalence of amyloidosis of 5.8%, whereas other studies indicate a prevalence that ranges from 3 to 23% [14, 15]. The prevalence may be around 5% in patients with ankylosing spondylarthritis. In Turkey however, familial Mediterranean fever and tuberculosis are more prevalent causes of amyloidosis than rheumatoid arthritis [12]. Bronchopulmonary infections remain a cause of amyloidosis and cystic fibrosis has also been rarely reported. Among tumours, hypernephroma is no more the internist's tumour with its cluster of systemic manifestations, including AA amyloidosis, as it is now easily and early diagnosed by ultrasonography or computed tomodensitometry. A lot of tumours are uncommon causes of AA amyloidosis, without a strong predominance of a specific histological type. However, we have to mention Castleman's tumour as one of the more recently recognised cause of amyloidosis. This deserves to be known, as tumour resection in the unicentric form of this disease, can lead to the regression of clinical signs of amyloid nephropathy [16].

Diagnostic and Evaluation of a Patient with Amyloidosis

Amyloidosis as a Great Masquerader

Clinical signs of amyloidosis are multiple as most organs can be involved and for some of them several signs are known (Table 3). However, few signs are specific or pathognomonic of amyloidosis. Most are not specific and the diagnosis is rarely suspected in view of a unique sign. Instead, multiple signs often suggest the diagnosis of another disease mimicked by amyloidosis. In view of this last property, amyloidosis can be considered as a new great masquerader (Table 4). In fact the diagnosis needs to be considered more often when there is a combination of non specific signs or when a sign is present in a clinical context compatible

Table 2. The Main Causes of AA Amyloidosis in Four Series of Live Patients

| Period | Brandt 1968 | Gertz 1991 | Browning 1985 | Joss 2000 | Tuglular 2002 |
|----------------|-------------|------------|---------------|-----------|---------------|
| | 1960-1966 | 1956-1989 | 1973-1982 | 1985-1999 | ? |
| Country | USA | USA | UK | UK | Turkey |
| Infectious | 10 (43%) | 11 (17%) | 13 (17%) | 6 (14%) | 53 (20%) |
| Tuberculosis | 7 | 0 | 8 | 6 | 28 (10%) |
| Bronchiectasis | 1 | 5 | 2 | | 18 |
| Osteomyelitis | 2 | 5 | 3 | | 7 |
| Other | 0 | 1 | 0 | | |
| Rheumatic | 8 (35%) | 42 (66%) | 56 (74%) | 30 (70%) | 30 (10%) |
| RA | 8 | 31 | 47 | 22 | 12 |
| Ank. Spond. | 0 | 5 | 6 | 5 | 10 |
| JIA | 0 | 1 | 2 | 3 | 0 |
| Psor. Arthr. | 0 | 3 | 0 | | 0 |
| Other | 0 | 2 | 1 | | 8 |
| IBD | 1 (4%) | 6 (9%) | 3 (4%) | 1 (2,5%) | 0 (0%) |
| Malignancies | 3 (13%) | 2 (3%) | 3 (4%) | 1 (2,5%) | 0 (0%) |
| Miscellaneous | 1 (4%) | 3 (5%) | 1 (1%) | | 6 (2%) |
| FMF | 1 | 2 | 0 | | 183 (64%) |
| Idiopathic | 0 | 1 | 1 | 5 (11%) | 17 |
| Total Number | 23 (100%) | 64 (100%) | 76 (100%) | 43 (100%) | 287 (100%) |

RA = rheumatoid arthritis; Ank. Spond. = ankylosing spondylitis; JIA= juvenile idiopathic arthritis; Psor. Arthr. = psoriatic arthritis; IBD = inflammatory bowel diseases; FMF = familial mediterranean fever.

Table 3. Clinical Signs of Systemic Amyloidosis

| Signs | Frequent | Uncommon or rare |
|------------------------|--|---|
| kidney | proteinuria, nephrotic syndrome renal failure | renal vein thrombosis tubular disorder |
| heart | heart failure conduction and rhythm disturbances | angina pectoris arterial emboli valvular disease pericarditis right ventricular rupture |
| gastrointestinal tract | macroglossia, dysphagia, abdominal pain vomiting, hemorrhage, pseudoobstruction malabsorption, perforation | pancreatic involvement colonic ulceration bowel infarction |
| liver | hepatomegaly | portal hypertension ascites, jaundice liver rupture |
| spleen | splenomegaly | hyposplenism spleen rupture |
| peripheral nerve | sensory and autonomic polyneuropathy | cranial neuropathy |
| skin | petechiae, purpura, ecchymoses nodule, papule, plaque | scleroderma- like lesion bullous lesion onychodystrophy alopecia |
| respiratory tract | cough, hemoptysis | dyspnea |
| bone and joints | polyarthropathy carpal tunnel syndrome | polymyalgia rheumatica fracture spinal cord compression |
| endocrine glands | goitre | hypothyroidism adrenal insufficiency |
| urinary tract | hematuria, ureteral pain, obstruction | |
| eye | visual flutters | decrease of visual acuity, pain |
| lymph nodes | | hypertrophy |
| large vessels | | jaw claudication |

with the existence of amyloidosis. At present it is often evoked only when a potential cause of amyloidosis such as an inflammatory disorder (AA), a monoclonal immunoglobulin component (AL), or a history of hereditary disease is present.

General signs such as fatigue and weight loss are frequently reported in severe forms of the disease. Renal signs are the most frequent but also the least specific. Thus, if proteinuria is the principal mode of discovery of generalised amyloidosis, the clinical background is essential for the physician to consider the diagnosis. Persistent nephrotic syndrome accompanied with advanced renal failure and enlarged kidneys can be considered as relatively suggestive of amyloidosis; although this association may exist in some types of glomerulonephritis.

Cardiac involvement gives relatively consistent signs including heart failure, arrhythmia or conduction disturbances. Other signs which are less common include : angina pectoris, systemic arterial emboli, pericarditis, mitral regurgitation, right ventricular rupture. Hepatic enlargement is common but complications such as portal hypertension, ascites, jaundice, liver rupture, are rare. Splenic enlargement is also common, hyposplenism and splenic rupture are rare. Digestive tract symptoms are many, reflecting the different levels of anatomical lesions: dysphagia, abdominal pain, vomiting, hemorrhage, diarrhea, pseudoobstruction and true occlusion, perforation, intestinal infarction. Nerve involvement is

essentially a sensory and motor polyneuropathy starting in the lower limbs. Cranial nerves are rarely affected. Amyloidosis is an established cause of the carpal tunnel syndrome. Muscle involvement with pseudohypertrophy and functional impairment is uncommon.

Skin lesions are highly polymorphic: common lesions are: petechiae, purpura and ecchymoses, papules, nodules and plaques; uncommon lesions are: scleroderma-like diffuse infiltration, bullous lesions, onychodystrophy, alopecia. Cough, hemoptysis, dyspnea can reveal the various types of respiratory tract lesions: bronchial, mediastinal and parenchymatous. Joint amyloidosis can present as a symmetric polyarthropathy involving shoulders with the classic shoulder-pad sign, wrists, hands and knees. Rarely, it may present as a polymyalgia rheumatica. Spinal cord lesions are uncommon. Fractures secondary to destructive bone lesions have been reported. Pain and hematuria can be signs of ureteral and bladder amyloidosis. All areas of the eye can rarely be involved by amyloid deposits: eyelid, conjunctiva, cornea, orbit. Vitreous deposits can mimic uveitis.

How to Diagnose Amyloidosis ?

The diagnosis of systemic amyloidosis still relies on biopsy showing amyloid deposits. Two strategies are possible for the biopsy. First, it can be taken from a clinically involved organ. Second, it can be taken from a clinically uninvolved organ, but one known to frequently contain amyloid deposits in the systemic forms. In the first case, the biopsy is not innocuous

Table 4. Amyloidosis as a Masquerading Syndrome

| Organ | Symptom or sign | Masquerade syndrome |
|---------------------------|--|---|
| heart | Q waves | myocardial infarction |
| gastrointestinal tract | sicca syndrome dysphagia colonic ulcerations | Sjögren syndrome esophageal cancer ulcerative colitis |
| liver | hepatomegaly | liver cancer |
| skin | digital infiltration | scleroderma |
| bone | lytic process | myeloma, metastases |
| joints | polyarthritits | rheumatoid arthritis |
| urinary tract | hematuria | ureteral or bladder tumor |
| eye | vitreous deposits | uveitis |
| lymph node | hypertrophy | lymphoma |
| vessels | jaw claudication | temporal arteritis |
| in any place: amyloidomas | mass syndrome | tumor |
| multiple lesions | organ infiltration | metastatic tumor |

and is often unpleasant for the patient. Rarely, the organ biopsy is easy to perform, as is the case for the skin. In general, biopsy of a clinically involved organ is to be performed as a second-line technique, and the second strategy should be favoured. This implies, however, that the diagnosis of amyloidosis be considered on clinical signs.

Currently, three types of biopsy are commonly performed: 1) biopsy of the gastrointestinal tract: rectal or gastroduodenal, 2) subcutaneous abdominal fat aspiration biopsy (AFA), 3) labial salivary gland (LSG) biopsy.

Gut Biopsy

Rectal biopsy was introduced in the sixties by the Tel-Hashomer hospital group in Israel [17]. Rectal biopsy is performed during a proctoscopy or sigmoidoscopy, and must include submucosal vessels, which are more likely to contain amyloid deposits than those of mucosa and muscularis mucosa. Many other series have found good sensitivity of this technique for the diagnosis of systemic AA, AL and ATTR amyloidosis. Since gastrointestinal vessels are all involved, amyloid deposits are also accessible with gastric or duodenal biopsy obtained during gastrointestinal endoscopy. Some data suggest that gastroduodenal biopsy could be as informative as rectal biopsy [18].

Abdominal Fat Aspiration

Abdominal fat aspiration was introduced in the early seventies by Per Westermark [19]. He showed that amyloid deposits were frequent in the subcutaneous tissue taken at post mortem from patients with AA amyloidosis. These deposits were mainly around adnexal structures, often making a ring around adipocytes [20]. He also noticed that the highest density of amyloid deposits was in the scalp and in the abdominal wall. The original technique described by Westermark appears easy [19, 21]. This technique is easy, not painful when using a local anesthesia. It can also be performed in case of hemostasis abnormalities [22]. The sensitivity of AFA is similar to rectal biopsy, ranging from 55% to 75%. Many studies highlighted its value for the diagnosis of AA amyloidosis, as well as for AL and ATTR amyloidosis [23-25]. On the other hand AFA does not appear to be sensitive for the diagnosis of A β 2M [21, 26]. Most of these results are from tertiary centers, drawing from a population with a high prevalence of patients with amyloidosis, and whose pathologists are very experienced in amyloidosis [27]. It is likely that these very favourable results for AFA cannot be extended without caution to less specialized centers. A study emphasizes the possibility of false positive results with AFA due to collagen fibers [28]. These authors describe the points to distinguish amyloid from collagen and remind us that AFA belongs to cytology and deserves active participation by pathologists [29, 30]. Some authors do the subcutaneous biopsy with a Tru-cut® needle and have good results [31, 32]. Standard skin biopsy has not been extensively evaluated for the diagnosis of amyloidosis. One group reported satisfactory results with a skin biopsy of the forearm [33]. The skin biopsy may be more useful in hereditary amyloidosis [34].

Labial Salivary Gland Biopsy

The third simple method for the diagnosis of amyloidosis is biopsy of accessory salivary glands, situated in the labial mucosa. It now replaces the old gingival biopsy. This latter was often used in the fifties, when renal biopsy was at its beginning and rectal biopsy not yet considered for diagnosis [35]. However, this biopsy was unpleasant and its sensitivity very variable. Recently, Delgado and Mosqueda, inspired by some isolated cases of amyloidosis revealed by a sicca syndrome [36] reported a comparative study of gingival and LSG biopsy [37]. In this series of 19 patients with a clinical suspicion of AA amyloidosis, LSG biopsy was positive in 19 cases (100%), whereas gingival biopsy was positive in only 3 cases (16%). This preliminary work on LSG biopsy was extended to a series of 30 patients with biopsy proven AA and AL-amyloidosis [38]. LSG biopsy can also be used for the diagnostic of ATTR amyloidosis [39].

How to Choose Between These Techniques ?

There is no study comparing rigorously these techniques. In particular, in series evaluating AFA, the gold standard biopsy varies from one series to the other. One series aimed at comparing AFA, gastrointestinal and renal biopsies in patients with rheumatoid arthritis. It suggested that results of gastrointestinal biopsy are highly correlated with those of renal biopsy, but the results of AFA are not [40]. It is likely that the experience acquired by the team of physician-pathologist with one technique is probably the main factor for success regarding the diagnosis of amyloidosis. When these indirect techniques do not provide the diagnosis, direct biopsy of the involved organ can be considered. Renal biopsy is rarely negative; in one series it was positive in 94% of 81 patients with AL amyloidosis [41]. Nevertheless, renal biopsy is not without risk and is relatively painful.

Liver biopsy is hazardous, and although some series do not report any severe complications [41], other old series report fatal hemorrhagic events [35]. Thus it should be avoided, despite its great sensitivity. Sural nerve biopsy is painful, and as neuronal amyloid deposits are patchy, not very sensitive [42]. Endomyocardial biopsy is an invasive procedure that should be performed only in isolated cardiac forms, where it will be very informative [41]. The bone marrow biopsy, which is almost systematically taken to look for a plasmacytic disease when the diagnosis AL amyloidosis is suspected, is reported to have amyloid deposits in about half of the cases of AL amyloidosis [41].

What is the Nature of Amyloid Deposits ?

Characterization of the biochemical nature of amyloid deposits is indispensable in order to establish a thorough diagnosis and prognosis and to propose appropriate treatment. In most of the cases, the clinical context is informative and the diagnosis of one of the three forms of multisystemic amyloidosis AL, AA or ATTR can be directly made.

Clinical Diagnosis is Often Easy but Holds Some Pitfalls

Following data are of value for the diagnosis of AL amyloidosis: 1) A plasmacytic disease, most often multiple myeloma, is already known, or is discovered at the time of presentation. 2) A monoclonal component in the serum or urine 3) Evidence of involvement of heart, skin, peripheral nerve, carpal tunnel, tongue with macroglossia, joints, and factor X deficiency. On the other hand, kidney, liver, spleen, and gastrointestinal tract involvement are common to AL and AA amyloidosis.

Of utmost importance for the diagnosis of AA-amyloidosis is the existence of a disease known to lead to this complication. Usually it is a long standing inflammatory disease. However, when the underlying disease is a cancer, it may be previously undiagnosed, and discovered only at the same time as the amyloidosis. There are also some cases of AA-amyloidosis with no evidence of underlying inflammatory disorder [43].

Last, a family history of similar disease should be investigated in every patient with amyloidosis, mainly when there is peripheral neuropathy, but also in the case of renal, cardiac, cutaneous or ocular disease.

Sometimes, it is difficult to make a definitive diagnosis from clinical data, and immunohistochemistry is needed to determine the type of amyloidosis. Some of these clinical situations are quoted as examples: 1) Several organs can be affected in two different types of systemic amyloidosis: kidney, liver, gastrointestinal tract, and spleen may be involved in AL and AA-amyloidosis; heart, peripheral nerve, gastrointestinal tract, and kidney in AL and ATTR amyloidosis. 2) The presence of a monoclonal component does not absolutely imply its role in the formation of amyloid deposits. Indeed some inflammatory diseases can be associated with a monoclonal component and complicated with AA-amyloidosis [44] or AL-amyloidosis [45]. Moreover, the prevalence of a monoclonal component increases with age and may be associated fortuitously with a non AL-amyloidosis. 3) Some inflammatory or tumoral diseases can be associated with either AA or AL-amyloidosis. This is the case for multiple myeloma and renal adenocarcinoma which are usually associated with AL and AA-amyloidosis respectively, but which were described in some cases to be associated with AA and AL respectively [46, 47]. 4) In hereditary amyloidosis, the diagnosis of neuropathy has not always been made in the parents and another diagnosis has sometimes been reported: multiple sclerosis, syringomyelia, Charcot's disease, "myopathy" and so on. The hereditary nature can even be completely masked, as the penetrance of the disease is variable and many cases of hereditary amyloid neuropathy appear sporadic. In one series, 30 % of patients with amyloid polyneuropathy due to TTR were treated for AL-amyloidosis [48]. ATTR amyloidosis is probably under diagnosed, not only in neurologic forms, but also in cardiac and ocular forms.

The main diagnosis pitfall is, when evidence for a circulating monoclonal component is lacking, to distinguish AL from ATTR when the disease presents as a neuropathy and/or cardiopathy, and AL from fibrinogen amyloid (Afib) when the clinical presentation is restricted to nephropathy [49]. This could be improved by the development of a very sensitive nephelometric method for quantitating free light chain of immunoglobulin, which could be particularly useful in patients with negative immunofixation results for serum, urine, or both [50].

Besides immunohistochemistry, the potassium permanganate method helps to characterize amyloid proteins [51]. The sensitivity of amyloid proteins to permanganate, as shown by loss of Congo red affinity and altered birefringence, is specific to AA and A β 2M amyloid, whereas AL and ATTR amyloid are resistant to such treatment. This method is on the whole well correlated with immunochemistry, but its interpretation is

sometimes difficult especially when amyloid deposits are extensive and the sensitivity to permanganate incomplete; In addition it allows for distinction of only two groups of amyloidosis [52]. Practically, the permanganate method permitted distinction between AA and AL amyloidosis, but it is now supplanted by immunohistochemistry.

Immunohistochemistry is the Modern Way to Type Amyloid

This typing can be performed for every tissue obtained for the diagnosis: kidney, gut, LSG biopsies and even AFA [21, 53]. Immunohistochemistry which includes immunofluorescence and immunoenzymatic methods, allows study of amyloid deposits with antibodies directed against the known amyloid proteins: immunoglobulin light chains, AA protein, TTR, Aβ protein, β2microglobulin or even apolipoprotein A1 apolipoprotein A2, gelsolin, fibrinogen Aα chain and lysozyme. For the typing of systemic amyloidosis, it is usually sufficient to use antibodies directed against immunoglobulin light chains, protein AA and TTR. Antibodies to protein AA are easy to use and recognize the great majority of deposits made of protein AA; although protein AA and its fragments are of variable length. Commercial antibodies to light chain immunoglobulins react with a lower proportion of AL deposits, as these proteins are more variable, and especially a subgroup of light chains is not recognized [21]. Moreover, for some authors, AL deposits are damaged by usual fixation, and immunochemistry with frozen tissue is more reliable [54]. Antibodies to TTR, which are of good quality, should be more routinely used, because, as cited earlier, the clinical presentation of ATTR amyloidosis is diverse and immunochemistry helps to recognize these forms. Some TTR variant proteins can also be detected in the plasma of the patient by electrophoretic methods which are not yet routinely used [55].

Finally, the typing of amyloid can be done by chemical methods based on water extraction, purification and sequencing of the protein [56]. Micromethods allowing amyloid protein purification from very small deposits have been more recently developed [57]. Amyloid extraction can also be followed by an ELISA test with antibodies against the usual amyloid proteins. These methods are also not yet routinely used.

Extent of Amyloidosis

The extent of the disease is obviously a critical point, as it governs the prognosis. Clinical evaluation is to be performed systematically to determine as finely as possible the degree of organ involvement. Some organs are preferentially the place of localized deposits; skin, bladder and ureter, ocular conjunctiva, larynx, trachea and bronchial tube. However, it is important to determine that, even in these cases, amyloidosis is not generalized.

Physical examination remains the cornerstone of this evaluation, searching for the various signs described earlier. Some easy and innocuous complementary tests can be performed systematically, others are to be discussed in accordance with clinical examination and first line procedures (Table 5). Amyloid P-component scintigraphy will perhaps become a standard technique in the future, but its availability remains restricted to a few centres and it entails some risk [58].

When the diagnosis of amyloid is made, is it useful to take a biopsy of each organ exhibiting signs of amyloid involvement? Practically, the answer will be no, if clinical signs and complementary tests are in agreement with the diagnosis of amyloidosis. Nevertheless, multiple biopsies may be proposed in certain cases. For example, in a patient with amyloid deposits in a rectal biopsy, clinical renal signs suggest the presence of renal amyloidosis. However other nephropathies can be evoked in accordance with the clinical context: drug nephropathy, specific rheumatoid arthritis nephropathy, vasculitis, and thus a kidney biopsy may sometimes be performed to verify the diagnosis.

AMYLOIDOSIS AS A COMPLICATION OF AUTO-INFLAMMATORY SYNDROMES

As stated above, the etiology of AA amyloidosis, one of the three main types of multisystemic amyloidosis, has been changing. In this type, the amyloid protein, named AA protein, is derived from the serum amyloid A (SAA) protein, which belongs to the group of acute phase proteins . In fact, amyloidosis may appear as a complication of most diseases associated with a persistent inflammatory response, whatever the primary cause: infection, tumor or inflammation. In some series, FMF appears as the main cause of AA amyloidosis. FMF is now recognised as belonging to a member of a group of diseases called hereditary periodic fever syndromes or auto-inflammatory syndromes [59]. Three other diseases presenting mainly as intermittent bouts of inflammatory symptoms have been so far clinically as well as genetically characterised: Tumor Necrosis Factor receptor superfamily 1A Associated Periodic fever Syndrome (TRAPS), hyperimmunoglobulinemia D and periodic fever syndrome (HIDS), and the most recently recognised entity which includes Muckle Wells syndrome, familial cold urticaria, and the Chronic Infantile Neurological Cutaneous and Articular (CINCA) also known as Neonatal Onset Multisystemic Inflammatory Disease (NOMID) syndrome (Table 6).

A thorough diagnosis, which now relies on combined clinical and genetic data, is warranted because of specific clinical and therapeutic management of each of these four diseases. Intriguingly, all auto-inflammatory syndromes, can be complicated by AA amyloidosis.

Clinical Aspects of AA Amyloidosis

There is a Preclinical Phase in AA Amyloidosis

This phase is defined by the formation of amyloid deposits in tissue, without any clinical manifestation. Obviously, it is difficult to evaluate the natural history of amyloid deposition and to know the length of this phase and its final outcome. It is unknown whether all patients with longstanding inflammation and silent amyloid deposits will always develop overt symptoms related to amyloid deposits with time or whether deposits will remain silent in some. In fact, in a cohort study of patients with rheumatoid arthritis showed that amyloid fat deposits are not uncommon (16.3%). However, in the majority of patients, the deposits did not indicate clinically evident organic dysfunction, even after several years of follow-up. Patients with more extensive fat deposits had a higher risk of developing

Table 5. Amyloidosis Extension

| Complementary tests | Physical examination | |
|---|---|---|
| | systematic | optional |
| Kidney | proteinuria serum creatinine ultrasound | renal vein ultrasound |
| heart | X-ray film of the chest ECGechocardiography | 99m Tc pyrophosphate scintigraphy Holter monitor |
| gastrointestinal tract | serum protein electrophoresis hepatic enzymes | esophageal manometry |
| liver | ultrasound | liver/spleen scan |
| spleen | ultrasound hemogram | indicating size and function of spleen peripheral smear for Howell-Jolly bodies |
| nerve | | electromyogram |
| respiratory tract | X-ray film of the chest | blood gases bronchial endoscopy thoracic |
| endocrine glands | corticotropin stimulation test TSH | |
| hemostasis | Quick time, APTT serum fibrinogen | |
| Global amyloid burden and organ specific deposition | | Amyloid P-component scintigraphy |

Table 6. Characteristics of the Four Main Types of Hereditary Fevers

| | FMF | HIDS | TRAPS | M-W_FCAS/CINCA |
|---------------------|---|---|--|--|
| Mode of inheritance | recessive | recessive | dominant | dominant |
| Age at onset | <20 years | childhood | variable | childhood/neonatal |
| Length of the | 1-4 days | 3-7 days | often more than 1 up | variable |
| Access | to several weeks | | | |
| Abdominal pain | very common (serositis) | very common | common | rare |
| Musculoskeletal | monoarthritis | athralgias | myalgias | arthritis/destruction |
| Chest pain | pleuritis, often unilateral | unusual | yes | no |
| Rash | rare (<5%) erysipelas-like on lower limbs | very common (>90%) or several types: maculopapular, papular | common erysipelas-like including upper limbs | urticaria/erythema |
| Other signs | pericarditis, scrotal attacks, splenomegaly | headache cervical lymph nodes hepatosplenomegaly | orbital edema | deafness cold sensitivity/dysmorphism papillitis |
| Amyloidosis | yes | yes | yes | yes |
| Treatment | colchicine | TNF inhibitors | steroids | steroids |
| | | Statin? | TNF inhibitors | Interleukin-1 inhibitor? |
| Chromosomal locus | 16p13.3 | 12q44 | 12p13 | 1q44 |
| Gene | <i>MEFV</i> | <i>MVK</i> | <i>TNFRSF1A</i> | <i>CIAS1</i> |
| Protein | marenostrin/pyrin | mevalonate kinase | type 1 TNF receptor (55p) | cryopyrin |

clinical amyloidosis [60]. Serum amyloid P component scintigraphy probably may also help in an early diagnosis of amyloidosis [58].

Nephropathy is the Predominant Clinical Manifestation

Proteinuria remains the first detectable sign of AA amyloidosis. Patients with longstanding inflammatory disorders should be regularly screened for proteinuria to detect renal involvement at an early stage. Unfortunately renal amyloidosis remains often discovered in presence of the nephrotic syndrome, as observed in 88 % of the patients in a recent series from Turkey [13]. Nothing has been heretofore published on the detection with microalbuminuria of AA amyloid nephropathy. A recent study suggests however that microalbuminuria could be an early marker to detect renal amyloidosis in hereditary transthyretin amyloidosis [61]. Other organ involvement is classical: digestive signs related to every part of the gut, splenomegaly, and hepatomegaly with cholestasis, goitre, and lately cardiopathy. Staining amyloid deposits with antibodies directed against AA amyloid protein is currently the routine histological way to diagnose AA amyloid [62].

When overt renal disease is present, the outcome of patients with AA amyloidosis is bad with a five-year patient survival around 40% [11, 12]. In one series, the prognosis of AA amyloid is not better than the prognosis of AL [11].

SAA as the Precursor of the Amyloid Protein

Serum Amyloid A, the Highly Polymorphic Precursor Protein, is an Acute Phase Protein

Shortly after the characterisation of the unique nature of AA amyloidosis a serum component was detected which reacted with antibodies raised against the amyloid AA protein. This serum amyloid A protein appeared to be an apolipoprotein associated with HDL and a generally present constituent of acute phase serum. SAA is a very sensitive acute phase protein which increases 100 to 1000 fold after tissue damage or infection and is mainly produced by the liver.

Serum SAA levels are increased in a number of chronic inflammatory and neoplastic diseases which may predispose to amyloidosis. In hepatitis, ulcerative colitis, and connective tissue diseases there is a relative deficiency of SAA production. Bacterial infections generally induce higher SAA serum levels than viral or parasitic infections. SAA levels are elevated in patients suffering from different types of malignancies, with significantly higher levels in patients having metastatic and advanced disease [63]. Measurement of SAA has been standardized recently [64]. Serum concentration of SAA directly influences the amyloid load as assessed by amyloid P component scintigraphy [65].

The molecular weight is 12.5 kDa and the protein is composed of 104 amino acids. Human AA usually has 76 amino acid residues and is derived from SAA by proteolytic removal of residues 77-104 at the C-terminus [63]. The 76 amino acids AA protein has been rarely isolated in the plasma and when and where the cleavage happens has not been thoroughly determined [66]. Cathepsins, a family of lysosomal thiol proteases, may be the enzymes involved in the processing.

The three human SAA genes (SAA1, SAA2, and SAA4) are tightly linked and located on chromosome 11. The fourth locus, SAA3, is a pseudogene. The different alleles of the acute phase SAA1 (SAA1.1, SAA1.2, SAA1.4 SAA1.4 and SAA1.5) and SAA2 loci (SAA2.1 and SAA2.2) encode different isoforms. Because of the enzymatic removal of the N-terminal arginine by hepatic aminopeptidase two isoforms with a different iso-electric focusing point per single gene product (i.e. the complete protein and the des-arg form) can be detected in the serum. SAA2.1 and SAA2.2 differ only at position 71, and both proteins differ from SAA1 by at least 7 amino acids. SAA4 differs considerably from the other two SAA subtypes and shares only 55% identity. This protein consists of 112 instead of 104 amino acids due to an octapeptide insert [67].

SAA4 is a normal apolipoprotein component of lipoprotein in the non-acute phase situation. Therefore this SAA has been designated constitutive SAA (C-SAA) to distinguish it from the acute phase proteins SAA1 and SAA2. In human hepatocytes and hepatoma cell lines acute phase proteins such as SAA are induced by interleukin-1 (IL-1), IL-6, IL-11, oncostatin M, leukemia inhibitory factor, ciliary neurotropic factor, transforming growth factor β and tumor necrosis factor and glucocorticoids [68]. Three general types of regulatory elements are responsible for cytokine-induced transcription: the binding site for IL-6, for the NF- κ B/Rel family, and for members of the C/EBP transcription factor [69].

SAA Function

SAA can be found in many species, and thus probably will have one or more important biological functions. Since an increase in SAA is always seen after tissue destruction, a role of SAA in normal tissue repair has been suggested. Some of the proposed functions of SAA are: it may influence lymphocytic responses to antigens, induce T lymphocyte migration and adhesion, induce adhesion of resting CD4+ T cells after binding to specific extracellular matrix glycoproteins, have attractant activity for monocytes and polymorphonuclear cells, inhibit the oxidative burst response in neutrophils, inhibit platelet aggregation, and stimulate IL-8 secretion [70]. SAA may also appear to be an interesting link between inflammation and atherosclerosis. The fact that SAA is an apolipoprotein of HDL with displacement of apoAI from the HDL during inflammation points to a role of SAA in the lipoprotein metabolism.

A recent study reports that a recombinant acute phase isoform variant of human SAA 1.1 readily forms ion-channels in planar lipid bilayer

membranes at physiologic concentrations. This type of channel would place a severe metabolic strain on various kinds of cell and thus be related to a host defence role of acute phase SAA isoforms and may also be the mechanism of end organ damage in AA and other amyloidoses [71].

Familial Mediterranean Fever-Associated Amyloidosis

Familial Mediterranean fever-associated amyloidosis served to describe amyloid nephropathy as defined above. There is no data supporting that tissue distribution of amyloidosis deposits is specific in FMF-associated amyloidosis, however it has been suggested that abdominal fat aspiration was not as useful as rectal biopsy in this settings [72]. Amyloid nephropathy was the cause of death in FMF, before the colchicine era. In the absence of diagnosis of FMF and of appropriate treatment, amyloidosis remains a prevalent complication of FMF, as it is regularly reported in the Turkish population, including children [73].

In all forms of hereditary fevers there is a major inflammatory reaction during the crises with elevated serum levels of SAA and CRP [74, 75, 77, 78]. Present data on susceptibility factors to amyloidosis in recurrent hereditary fevers originate essentially from studies on amyloidosis in FMF, which was a lethal complication before the colchicine therapy era, and remains the main cause of amyloidosis AA in a few countries, like in Turkey [13]. All patients with FMF do not develop amyloidosis. Amyloidosis generally occurs in patients with early and severe inflammatory crises (phenotype 1). However, amyloidosis may occur in other circumstances, and even in patients with no clinical inflammatory crisis; this latter situation has been referred as phenotype 2 of FMF amyloidosis by Israeli authors. Phenotype 2 is certainly rare, as shown in a Turkish study that systematically investigated proteinuria in relatives of patients with FMF amyloidosis [78]. It probably results from the existence of blood inflammation between the crises. The serum amyloid protein (SAA) is the most sensitive marker of inflammation between clinical crises. In a series of 170 patients with FMF, the serum SAA mean value between crises reached 74.6 mg/l (N<10 mg/l) [79]. It is tempting to speculate that a similar infra-clinical inflammation is present in patients with phenotype 2. The distribution of MEFV gene mutations in these patients is similar to that of patients with the classical form of FMF [80, 81].

It has long been established that the prevalence of amyloidosis varies according to ethnic groups. This suggests that genetic and/or environmental factors participate in the occurrence of amyloidosis during FMF [82]. Several correlation studies between phenotype (presence or absence of amyloidosis) and genotype at the MEFV locus have shown the preferential association between renal amyloidosis and the M694V mutation in the homozygous state, at the patients' population level [80, 82-84]. However, this association is not exclusive, since at the individual level, all patients with this genotype do not develop amyloidosis. Conversely, all patients with amyloidosis do not present this genotype, more than 10 other genotypes having been associated with amyloidosis [82].

Among modifier genes that could influence the risk of occurrence of amyloidosis, genes encoding SAA proteins are the first candidates. Two genes encode the proteins, but several polymorphic variants have been described. A British study on patients with rheumatoid arthritis (RA) or chronic juvenile arthritis—two multi-factorial diseases with a genetic component, and characterized by a chronic inflammatory syndrome—has shown that the homozygous SAA1.1 genotype is more frequent in patients with secondary amyloidosis than in those without this complication [85]. Similarly, in patients with FMF, the homozygous SAA1.1 genotype is associated with an increased risk of amyloidosis, compared with other genotypes at the SAA1.1 locus. This result, which was initially obtained in the patients living in Armenia [86], has since then been confirmed in other populations with FMF [84, 87]. The risk of patients with the homozygous SAA1.1 genotype to develop amyloidosis relative to patients with other genotypes (odds ratio) is 7 in the Armenian population [86], 3 in the Israeli population [84], and 2.5 in the Turkish population [87]. It is, however, striking that in Japanese patients with RA, the risk of developing amyloidosis is associated with a different genotype (SAA1.3/SAA1.3) [88, 98]. A recent Americano-Japanese study [90] could reconcile this apparently contradictory results. Moriguchi *et al.* [91] found an association between a SNP located in the SAA1 promoter region (T at nucleotide position -13) and the risk to develop amyloidosis among Japanese patients with RA; it was suggested that, in this population, the -13T variant could be associated with the SAA1.3 allele [91]. Yamada *et al.* showed that the -13T nucleotide variation of the SAA1 promoter region is actually associated with amyloidosis not only among Japanese patients, but also

among patients of Caucasian origin living the U.S.A. [90]. In addition, according to this latter study, the -13T variation is frequently associated with the SAA1.3 allele in the Japanese population. However, such an association of the -13T allele with amyloidosis was not found in the Israeli population of patients with FMF [84]. In addition, another Japanese study indicates that the individuals carrying the SAA1.5 allele, which is not frequently associated with the -13T allele, have higher SAA plasma levels than individuals with another SAA1 genotype [92]. In summary, although most studies clearly identified the SAA1 locus as playing a major role in the risk to develop amyloidosis, the precise mechanism underlying this observation is still to be elucidated.

As shown in patients with FMF [84, 86], male sex is another factor that contributes to the risk of occurrence of amyloidosis, with a reported relative risk of 4 in the Armenian population [86], and 1.7 in the Israeli population [84]. Again, there is no straightforward explanation accounting for this observation. Although steroid hormones are known to modulate circulating levels of acute phase proteins [93], the inflammatory status of patients with FMF, according to sex, has not been studied.

Daily colchicine is an effective treatment to prevent attack's recurrence as well as amyloidosis [94]. The usual dose of colchicine is 1 mg/day, but this can be increased to 1.5 to 2.0 and sometimes 2.5 mg/day to control disease activity. In some cases, colchicine can make clinical signs of amyloidosis disappear. True non-responders to colchicine are very uncommon, most of them are non-compliant patients. Although some controversy exists as to the adverse effects of colchicine on sperm function, long term use of colchicine can be considered as globally safe including during pregnancy [95]. In these non-responders, no treatment has proven its efficiency. Interferon alpha has been proposed, but early promising results have not been confirmed [96].

TRAPS Associated Amyloidosis

Fever is constant during TRAPS attacks, which are generally at least of five days and up to three weeks duration, even though shorter attacks have been reported [97]. Abdominal manifestations, a wide spectrum of rashes (pseudo-cellulitis—the most distinctive lesion—, urticaria-like, generalised serpiginous plaques and patches) and painful, disabling, myalgias as well as thoracic and scrotal pain, periorbital edema and conjunctivitis can be observed in TRAPS attacks. As well as for FMF, long term prognosis of TRAPS can be jeopardised by the development of AA amyloidosis. About 20 % of the patients with TRAPS are estimated to develop this complication [76]. TRAPS associated amyloidosis could be determined in part by genetic factors. In fact, most cases of amyloidosis have been reported in-patients harbouring a cysteine residue mutation. However, this is not exclusive, as non-cysteine mutations can be associated with amyloidosis [98, 99]. Although there is no formal arguments, colchicine does not seem to prevent attacks recurrence in TRAPS patients. On the other hand, corticosteroids, when given at the onset, can attenuate the length and the severity of the attacks. In the most severe forms of the disease, clinical signs of inflammation are almost permanent and require daily use of corticosteroids. However, the efficiency of corticosteroid treatment seems to decrease in the course of the disease and higher and higher doses are necessary to obtain a clinical response [97].

According to the evidence of a proinflammatory effect of TNF during attacks, it is logical to propose TNF inhibitors as a treatment of TRAPS. Etanercept is a type 2 TNF receptor-immunoglobulin fusion molecule that mimics the effect of the normal soluble TNF receptor and thus seems particularly well designed to act in TRAPS. The posology of 25 mg SC twice a week is based on the experience acquired in rheumatoid arthritis, two subcutaneous injections each week. Preliminary results at 6 months in patients who use high doses of corticosteroids show an efficiency or etanercept, which induces a decrease of attacks frequency, and a decrease of corticosteroid dose [76]. Some TRAPS patients seem not to respond to etanercept [76, 100]. In one of these patients, another molecule consisting of the fusion of soluble TNFRSF1A with an immunoglobulin has been tested without any dramatic effect [100]. The effect of etanercept on the prevention of amyloidosis in TRAPS patients remains unknown. In one patient with probable amyloidosis, etanercept has induced a partial disappearance of the proteinuria [101] but in another patient, etanercept did not prevent this complication [76].

CIAS1 Associated Diseases and Amyloidosis

The *CIAS1* gene underlying Muckle-Wells syndrome, Familial cold urticaria (FCU) and CINCA syndrome encodes a protein named

cryopyrin/PYPAF1/NALP3 [102-105]. Muckle-Wells syndrome was primarily defined as the association of urticaria, renal amyloidosis and nerve deafness [102]. Its mode of inheritance is dominant autosomal. Apart from urticaria, inflammatory attacks consist of ocular signs such as conjunctivitis, and less frequently arthritis. Additionally, other signs are observed in some kindreds, papillary drusen, endocrine abnormalities, aphthous ulcers, abdominal hernias, dysmorphism, suggestive of certain degree of variability or the clinical expression. Among a number of types of familial urticaria, the cold induced form has specific features: a delayed onset, most often of few hours after exposure to a cold ambience, of urticaria with conjunctivitis, arthralgias and a moderate fever. This distinctive sign explains why it has been proposed to rename this variety Familial cold auto-inflammatory syndrome (FCAS).

CINCA syndrome has been distinguished from other forms of chronic arthritis in the child by Anne-Marie Prieur [103]. A neonatal onset is a unique feature of this syndrome. Fever is quite constant during attacks of the disease. Neonatal skin rash is usually a non-pruriginous diffuse urticarial erythema. A chronic aseptic neutrophilic meningitis is responsible for headache and severe central nervous system involvement during childhood. Articular involvement consists of arthralgias, and arthritis which are destructive and may lead to a disabling arthropathy during infancy, mainly of the knees, and bone involvement leads to a delay of bone growth. Ocular involvement consists of conjunctivitis, uveitis, and papillitis with optic atrophy which can lead to blindness. Bilateral neurosensory progressive hearing loss may occur. A distinctive more or less pronounced facial dysmorphism is almost constant : which gives to the patients a similar appearance.

Muckle-Wells and CINCA syndrome patients share some clinical signs, as do some patients with Muckle Wells and FCAS [106, 107]. These entities can lead to amyloidosis.

There is no efficient treatment of these diseases. Colchicine has sometimes some effect on the arthropathy of the Muckle-Wells syndrome. Intermittent or permanent corticotherapy is often used without modifying the course of the disease. Preliminary data suggest that novel anti-interleukin 1 antagonist anakinra could relief attacks of Muckle Wells patients and diminish amyloid related proteinuria [108, 109].

Future Anti-Amyloid Treatments

Specific anti-amyloid agents are currently developed. Small sulfonated molecules which inhibit the interaction between heparan sulfate-proteoglycan and the amyloid AA protein, have been able in an mouse model of AA amyloidosis to reduce the amyloid splenic burden [110]. One of these molecules is currently in the clinical trial phase. Other small molecules which can deplete serum of circulating amyloid P component have been recently used in a short series of amyloid patients with preliminary results [111]. Inflammation associated amyloidosis (AA amyloidosis) remains a long term but severe complication of chronic inflammatory diseases. It is therefore difficult to establish which treatment can at best prevent the development of amyloidosis. In case of overt amyloidosis, it is likely that potent anti-inflammatory agents that can keep SAA at a low serum level can halt the progression of the disease and potentially allow its regression. However controlled prospective trials are currently lacking to determine which is the best drug in this settings, even in rheumatoid arthritis which is the most frequent cause of AA amyloidosis. Additionally, novel specific anti-amyloid agents will appear in the next years as a complement of anti-inflammatory drugs.

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