SEIJA TIITINEN

Clinical Studies on Amyloidosis in Rheumatoid Arthritis

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ACADEMIC DISSERTATION

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CONTENTS:

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I . Tiitinen S, Myllykangas R, Helin H and Kaarela K (1988): Modern trends in the diagnosis of secondary amyloidosis. Scand J Rheumatol Suppl. 67: 30-31.

II . Tiitinen S, Kaarela K, Helin H, Kautiainen H and Isomäki H (1993): Amyloidosis -incidence and early risk factors in patients with rheumatoid arthritis. Scand J Rheumatol 22: 158-161.

III . Tiitinen S, Kaarela K, Filipowicz-Sosnowska A, Maczynska-Rusiniak B, Lehtinen K, Leirisalo-Repo M, Paimela L and Koskimies S (1992): HLA typing and seropositivity in Finnish and in Polish patients with rheumatoid arthritis and amyloidosis. Clin Rheumatol 11: 265-268.

IV. Maury CPJ, Liljeström M, Tiitinen S, Laiho K, Kaarela K and Ehnholm C (2001): Apolipoprotein E phenotypes in rheumatoid arthritis with or without amyloidosis. Amyloid: J Protein Folding Disord 8: 270-273.

V. Maury CPJ, Tiitinen S, Laiho K , Kaarela K and Liljeström M (2002): Raised circulating interleukin–18 levels in reactive AA amyloidosis. Amyloid: J Protein Folding Disord 9: 141-144.

VI . Tiitinen S, Kaarela K, Filipowicz-Sosnowska A and Jesien-Dudzinka E (1991): Treatment of amyloidosis with azathioprine or colchicine or methotrexate in patients with rheumatoid arthritis in Finland and Poland. Reumatologia 2: 138-143.

VII . Laiho K, Tiitinen S, Teppo A-M, Kauppi M and Kaarela K (1998): Serum C-reactive protein is rarely lost into urine in patients with secondary amyloidosis and proteinuria. Clin Rheumatol 17: 234-235.

VIII . Laiho K, Tiitinen S, Kaarela K, Helin H and Isomäki H (1999): Secondary amyloidosis has decreased in patients with inflammatory joint disease in Finland. Clin Rheumatol 18: 122-123.

ABBREVIATIONS

AA	Amyloid A
AADP	Amyloid A-degrading protease
ACE	Angiotensin-converting enzyme
AEF	Amyloid-enhancing factor
AFA	Abdominal fat aspiration
AL	Amyloid light-chain
AP	Amyloid P component
Apo E	Apolipoprotein E
APP	Acute-phase protein
AZA	Azathioprine
COL	Colchicine
CRP	C-reactive protein
CYC	Cyclophosphamide
DMARD	Disease-modifying antirheumatic drug
ESR	Erythrocyte sedimentation rate
GAG	Glycosaminoglycan
GFR	Glomerular filtration rate
GSTM	Gold sodium thiomalate
HB	Haemoglobin
HCQ	Hydroxychloroquine
HLA	Human leukocyte antigen
HDL	High-density lipoprotein
IL	Interleukin
JCA	Juvenile chronic arthritis

MBL	Mannan binding lectin
MTX	Methotrexate
NS	Not significant
NSAID	Non-steroidal anti-inflammatory drug
RA	Rheumatoid arthritis
RF	Rheumatoid factor
rSAA	Recombinant SAA
SAA	Serum amyloid A protein
SAP	Serum amyloid P component
SD	Standard deviation
SEM	Standard error of the mean
sTNF R1	Soluble tumour necrosis factor receptor type 1
TNF-α	Tumour necrosis factor-α

ABSTRACT

Rheumatoid arthritis (RA) is the leading cause of amyloid A (AA) in developed countries. From 1979 to 1985 amyloidosis was sought via rectal or gingival biopsy. In 1985 a new simple abdominal fat aspiration (AFA) technique was introduced. In 1987 this method had doubled the incidence of amyloidosis, found now at an earlier stage.

In an epidemiological 15-year follow-up examination, amyloidosis was found by AFA in six out of 74 patients still living (8%) of an original population of 102 with erosive and seropositive RA. Five of the 24 deceased patients had had amyloidosis. The 15-year incidence of amyloidosis in RA was thus at least 11% (11/102). Continuous and longstanding inflammatory activity was considered to be the principal risk factor underlying amyloidosis. Fewer patients developed amyloidosis when treatment was started with gold sodium thiomalate than with hydroxychloroquine.

HLA-A, -B, - C and-DR antigens were determined in 83 patients with RA and amyloidosis, 60 in Finland and 23 in Poland. There were no significant differences in the frequencies of HLA types between the RA patients with and without amyloidosis in either Finland or Poland, and no significant differences between the Finnish and Polish patients with amyloidosis. All the amyloidosis patients from Finland and 70% of those from Poland were rheumatoid factor positive.

The role of apolipoprotein E (Apo E) in the pathogenesis of amyloidosis was clarified.

The frequency of phenotypes among the RA and amyloidosis patients did not significantly differ from that in RA patients without amyloidosis or Finnish control subjects. However, the Apo E4 frequency among the RA patients without amyloidosis was significantly lower than that in Finnish control subjects. The presence of Apo E4 in a patient with RA could, however, represent a relative risk factor for developing amyloidosis.

Plasma interleukin-18 (IL-18) levels were significantly higher in RA patients with amyloidosis than in those without. This discrepancy was not due to differences in inflammatory activity, nor was it related to renal function.

In an open prospective study in Finland and Poland, 69 patients with RA and amyloidosis were treated with azathioprine (AZA) or colchicine (COL) or methotrexate (MTX). During a mean 26-month follow-up in Finland and a 23-month follow-up in Poland mean serum creatinine worsened significantly in the AZA groups. In Finland 15/24 had proteinuria of more than 0.1 g/l before and 13 after treatment. All 16 Polish patients had proteinuria (mean 2 g/l before and mean 6g/l after AZA treatment). In the Polish COL group (n=15) mean serum creatinine rose from 110 micromol/l to 129 micromol/l over 25 months, but mean urinary protein excretion remained the same, 2.2 g/l. In the Finnish MTX group

(n=14) over 21 months the erythrocyte sedimentation rate, the serum C-reactive protein (CRP) concentration and blood haemoglobin concentration improved significantly, but renal function did not deteriorate. Six patients had proteinuria before and four after treatment.

To assess the usefulness of CRP in proteinuric amyloidosis patients, 19 patients with proteinuria from 0.4 to 12.3 g/24h were examined. The serum CRP level did not correlate with excreted CRP when proteinuria was less than 8 g/24h. Under this limit CRP is a reliable measure of disease activity.

At the Rheumatism Foundation Hospital a study was made to establish whether a high incidence of amyloidosis is a consistent finding in patients with inflammatory joint disease. A total of 4508 biopsies were studied from 1987 to 1997. The annual number of new patients with amyloidosis decreased from 68 to less than 10. On the other hand, the activity of the hospital increased, as the number of annual hospitalisations doubled over the same period. It was suggested that a change in medication towards more frequent use of MTX may be the reason for the decreased incidence of amyloidosis in Finland.

Tiivistelmä

Nivelreuma on AA-amyloidoosin yleisin syy kehittyneissä maissa. Vuosina 1979-1985 amyloidoosia etsittiin peräsuoli- tai ienbiopsialla. Vuonna 1985 otettiin käyttöön uusi yksinkertainen vatsan ihonalaisen rasvakudoksen aspiraatio. Tällä menetelmällä 1987 oli amyloidoosin ilmaantuvuus kaksinkertaistunut ja amyloidoosi todettiin entistä aikaisemmassa vaiheessa.

Epidemiologisessa 15-vuotistutkimuksessa löydettiin amyloidoosia vatsan ihonalaisen rasvakudoksen aspiraatiolla 6:lta 74 potilaasta. Alkuperäisaineistoon kuului 102 erosiivista ja seropositiivista nivelreumaa sairastavaa potilasta. Seurannan aikana kuolleista 5:llä potilaalla oli todettu amyloidoosi. Täten amyloidoosin 15-vuotisilmaantuvuus oli 11% (11/102). Jatkuva ja pitkään kestävä tulehdus oli amyloidoosin selvä riskitekijä. Kulta- hoitoa saaneilla potilailla kehittyi selvästi vähemmän amyloidoosia kuin hydroksikloro-
kiinilla hoidetuilla.

HLA-A, -B,-C,-ja DR kudostyypit määritettiin 83 nivelreumaa ja amyloidoosia sairasastavalta potilaalta, 60:ltä Suomessa ja 23:lta Puolassa. HLA-tyyppien esiintyvyydessä ei ollut mainittavia eroja suomalaisten ja puolalaisten nivelreumaa ja amyloidoosia sairastavien potilaiden välillä. Kaikilla suomalaisilla ja 70%:lla puolalaisista amyloidoosipotilaista oli veressä reumatekijä.

Apolipoproteiini E:n (Apo E) osuutta tutkittiin amyloidoosin synnyssä nivelreumaa ja amyloidoosia sairastavilla potilailla. Apo E3/4 -fenotyyppi ei ollut lisääntynyt nivelreumaa eikä amyloidoosia sairastavien potilaiden välillä verrattuna suomalaiseen kontrolliaineistoon. Koska Apo E4:n esiintyvyys nivelreumapotilailla, joilla ei ollut amyloidoosia, oli pienempi kuin normaaliväestöllä, saattaisi Apo E4 olla amyloidoosin
riskitekijä.

Plasman interleukiini-18-pitoisuudet olivat merkitsevästi suuremmat amyloidoosipotilail-

la, kuin nivelreumaa sairastavilla potilailla, joilla ei ollut amyloidoosia. Eroon eivät vaikuttaneet tulehdusaktiviteetti eikä munuaistoiminta.

Avoimessa prospektiivisessa tutkimuksessa hoidettiin Suomessa ja Puolassa 69 potilasta, joilla oli nivelreuma ja amyloidoosi atsatiopriinillä (AZA) tai kolkisiinilla (COL) tai metotreksaatilla (MTX). Seurannan aikana Suomessa (26kk) ja Puolassa (23kk) seerumin kreatiniinipitoisuus suureni merkitsevästi AZA-ryhmissä. Suomessa 15:lla 24:stä oli proteinuriaa enemmän kuin 0.1 g/l ennen ja 13:lla hoidon jälkeen. Kaikilla 16:lla puola-

laisella potilaalla oli proteinuriaa (keskimäärin 2 g/l ennen ja 6 g/l AZA-hoidon jälkeen). Puolassa COL-ryhmässä (n=15) seerumin kreatiniinipitoisuus suureni arvosta 110 micromol/l arvoon 129 micromol/l 25 kuukauden aikana, mutta virtsan valkuainen säilyi keskimäärin tasolla 2.2 g/l. Suomessa MTX ryhmässä (n=14) 21 kk:n aikana lasko, seerumin C-reaktiivisen proteiinin (CRP) pitoisuus ja veren hemoglobiini paranivat huomattavasti, mutta munuaisten toiminta ei muuttunut, 6:lla potilaalla oli proteinuriaa ennen ja 4:llä hoidon jälkeen.

Arvioitaessa CRP:n käyttöä proteinuurisilla amyloidoosipotilailla, 19 tutkitulla protein-

uria vaihteli 0.4 –12.3 g/24 tunnissa. Seerumin CRP-taso ei korreloinut virtsaan eritty-

neeseen CRP:hen, kun proteinuriaa oli alle 8 g/24 tunnissa. Tällöin CRP:tä voidaan käyttää taudin aktiviteetin mittana.

Reumasäätiön sairaalassa suoritettiin 4508 biopsiaa erilaisia niveltauteja sairastaville potilaille vuosina 1987-1997. Tulokset osoittivat uusien yhden vuoden aikana tehtyjen amyloidoosidiagnoosien määrän laskeneen 68:sta 3:een, vaikka sairaalan toiminta saman aikavälin aikana kaksinkertaistui.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterised by chronic inflammation of the joints. This inflammation is mediated mainly by macrophages and proinflammatory cytokines, notably tumour necrosis factor-α (TNF-α) and cytokines of the interleukin (IL) family. Recent clinical trials have shown considerable improvement in patients treated with anti-TNF therapy.

AA amyloidosis is a serious extra-articular complication of RA and may result in significant morbidy and early death from organ failure. In AA amyloidosis insoluble fibrils are deposited in the spleen, kidneys, liver and gut, replacing vital tissue (Cunnane and Whitehead 1999). The acute-phase protein (APP) serum amyloid A protein (SAA), which circulates as an apolipoprotein of high-density lipoprotein (HDL), is the precursor of amyloid fibrils (Husebekk et al. 1985), and certain isotypes of SAA are more prone to form amyloid fibrils than others (Westermark et al. 1990). IL-1, IL-6 and TNF stimulate hepatocytes to synthesise SAA (Heinrich et al. 1990, Ganapathi et al. 1991).

The amyloid protein complex consists of two components: a fibrillar component with a polymeric antiparallel beta-pleated sheet molecular structure and a protein with pentagonal structure (pentraxin), the amyloid P component (AP). The extremely tight beta-pleated sheet configuration of the amyloid fibrils is responsible for the characteristic properties of amyloidosis: insolubility under physiological conditions, relative resistance against proteolysis, and its affinity for Congo red dye, yielding an apple-green birefringence under polarised light (Hazenberg and van Rijswijk 1994).

The present clinical study investigated the value of abdominal fat aspiration in detecting amyloidosis, and evaluated prognostic factors, heredity, treatment and the incidence of amyloidosis in RA. The prevalence of different apolipoprotein E phenotypes and the plasma levels of IL-18 were compared between RA patients with and without amyloidosis.

1 REVIEW OF THE LITERATURE

1.1. Other amyloidoses

According to Merlini and Bellotti 2003, 23 different proteins have been recognised as causative agents in amyloid diseases (Westermark et al. 2002). This dynamic process, which occurs as an alternative to physiologic folding, generates insoluble, toxic aggregates which are deposited in tissues in bundles of beta-sheet fibrillar protein. Amyloid deposits are identified on the basis of their apple-green birefringence under a polarised light microscope after staining with Congo red and the presence of rigid, nonbranching fibrils 7.5 to 10 nm in diameter seen on electron microscopy (Westermark et al. 2002).

Amyloid deposits are the basis of several conditions which have an enormous social and medical impact as well as being the cause of rare conditions which challenge the physician`s diagnostic capability. The deposition of amyloid in brain tissue underlies Alzheimer`s disease (Hardy and Selkoe 2002, Nussbaum and Ellis 2003), which affects more than 12 million people worldwide. The central nervous system is also the target of prior proteins, the cause of a group of rare hereditary or acquired neurodegenerative conditions (Prusiner 2001). The approximately 1 million patients who are receiving dialysis worldwide are at risk of symptomatic amyloidosis (Drueke 2000). The two most common forms of systemic amyloidosis are AL amyloidosis, with an incidence of approximately 1 case per 100.000 person-years in Western countries (Kyle et al. 1992), and AA amyloidosis due to chronic inflammatory diseases (e.g. RA and chronic infections). Hereditary amyloidosis constitutes an ever-expanding group of disorders which pose difficult diagnostic problems (Lachmann et al. 2002). The clinical features of systemic amyloidosis were reviewed by Falk and associates in 1997.

1 .2. Historical remarks on amyloidosis

Rokitansky mentioned amyloidosis as far back as 1842 (Rokitansky 1842, Husby 1985). The designation amyloid literally means ”starch”-like, referring to its typical staining properties with iodine-sulphuric acid as described by Virchow in 1854 (Cohen 1965). Histological definition became possible after Bennhold introduced the Congo red stain in 1922. Combined with polarisation microscopy this became the most useful test for the histological detection of amyloid deposits. Using electron microscopy, Cohen and Calkins (1959) disclosed the fibrillar nature of amyloidosis and described the unique morphological similarity among fibrils in different clinical settings. Benditt and Eriksen (1964) suggested for the first time that amyloidosis might be heterogeneous also with respect to the nature of amyloid fibrils. It was difficult, however, to isolate them from affected tissues due to their very low solubility in physiological conditions. Important progress was made by Pras and colleagues (1968), who published their water-extraction method for the purification of amyloid fibrils. This method permitted chemical and immunological analyses of isolated amyloid fibrils dissociated by treatment with agents such as sodium hydroxide, guanidine or urea (Husby and Sletten 1986, Husby 1992). This promoted the understanding of the chemical composition of amyloid.

Over the last 20-30 years it has become clear that amyloid fibrils may be composed of completely different protein subunits (Husby and Sletten 1986).

Histological proof of amyloidosis in patients may be obtained either by a direct approach such as renal biopsy (100% positive) (Kuhlbäck and Wegelius 1966) or by a general approach such as rectal biopsy (80% positive), gastric or duodenal biopsy (more than 90% positive), or labial salivary gland biopsy (86% positive).

Oral biopsy has been applied as a routine method at the Rheumatism Foundation Hospital in Heinola for diagnosing amyloidosis in patients with clinical suspicion (Sorsa et al. 1988). Amyloidosis may be an unexpected histological finding in biopsies of the liver, heart, gut,

peripheral nerves, lymph nodes, skin, thyroid gland or bone marrow, or indeed any tissue obtained at surgery (Gertz and Kyle 1989). Abdominal fat aspiration (AFA) is an easy and rapid procedure for policlinic patients, first introduced by Westermark and Stenkvist in 1973. Fixation in neutral buffered formalin and wax embedding are satisfactory for routine Congo red staining, but immunohistochemical staining reactions are intense on cryostat sections of fresh-frozen tissue (Kyle and Greipp 1983). It is of importance to ask the pathologist to examine RA patients specifically for amyloidosis, which should imply proper Congo red staining as recommended by Puchler and associates (1962) or staining with a recent modification of Eastwood`s Congo red method (Churukian and Schenk 1988) and investigation by polarising microscopy.

1.3. Aetiology

Raised SAA appears to be one of the prerequisites for the development of amyloidosis. Effective treatment of the underlying disorder, e.g. RA, which lowers levels of SAA , is recommended both as a prophylactic and therapeutic measure against amyloidosis.

Amyloid A-degrading protease (AADP) activity correlates positively with serum albumin levels in patients with RA complicated by amyloidosis. It has been suggested that the development of hypoalbuminaemia in patients with amyloidosis may give rise to a vicious circle leading to an accelerated reduction in AADP activity and accelerated amyloidogenesis (Maury and Teppo 1982).

During earlier decades, chronic infections such as tuberculosis and osteomyelitis were the main reasons for amyloidosis, but in developed countries, with a decline in chronic infections, RA has become the most common cause (Gertz and Kyle 1991).

1.4. Pathogenesis

Amyloid A (AA) is a 76-amino acid polypeptide with a molecular weight of 8.5 kD, which is derived from the NH2 terminal of the acute-phase reactant SAA. The molecular weight of SAA is about 12kD, corresponding to 102-104 amino acids. SAA is the most sensitive acute-phase protein yet characterised and complexed to high-density lipoprotein (HDL) as an apoliprotein in serum. An iso-electric focusing technique has shown that SAA constitutes a cluster of proteins of several isoforms (Maury et al. 1985, Yakar et al. 1995). In humans there are six possible isoforms (three to six in each individual) which are products of five SAA1 and two SAA2 gene alleles. Most SAA proteins in serum are the products of SAA1 genes.

In 1992, a novel SAA protein which is the normal apolipoprotein component of non-acute- phase lipoprotein was described (Whitehead et al. 1992). Because this SAA species is not significantly increased during the acute-phase response, it has been designated constitutive SAA to differentiate it from the SAA isoforms involved in this response. Constitutive SAA contains 112 amino acid residues, and its biological function remains to be elucidated. The SAA proteins are produced mainly in the liver, and the SAA level may increase up to 1000-fold during an inflammatory response. Extrahepatic formation of SAA may occur: transcription of SAA1 mRNA has been demonstrated in macrophages, endothelial cells, adventitial adipocytes and muscle cells derived from human atherosclerotic lesions (Meek et al. 1993). The average amount of SAA in the serum of healthy individuals is about 1 to 2 mg/l, with an upper normal level of 8 to 10 mg/l (Marhaug et al. 1994). SAA has a short half-life of 40 to 60 minutes. The lack of suitable methods for routine assay of SAA has been a problem; a rapid automated enzyme immunoassay for SAA was described by Wilkins and associates in 1994.

In the induction of SAA synthesis, several cytokines are involved, first of all IL-1, IL-6 and TNF, but the relative importance of these cytokines appears to be cell type-specific and to vary in various

experimental settings (Marhaug et al. 1994). At present, the biological function of SAA remains unclear. A role for SAA in cholesterol metabolism has been proposed by reason of the lipid-binding properties of SAA and because the protein is an inhibitor of the enzyme lecitin-cholesterol acyltransferase. SAA increases the affinity of HDL for macrophages and , possibly, all cells of the reticuloendothelial series, thereby serving to direct SAA-bound HDL to sites of macrophage accumulation. Such regions include the splenic perifollicular zone, hepatic sinusoids and glomerular mesangium, the precise sites at which AA amyloidosis forms (Kisilevsky and Young 1994).

Serum amyloid A also appears to modulate the inflammatory response. Human recombinant SAA (rSAA) is a chemoattractant; in concentrations similar to those found during the acute phase response, rSAA induces the directional migration of monocytes and polymorphonuclear leukocytes (Badolato et al. 1994).

An enzyme potentially important in the generation of amyloidosis protein from SAA is the lysosomal thiol protease cathepsin B. Digestion of recombinant human SAA1 with this enzyme and analysis of the products by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and aminoterminal sequencing has revealed that amyloid A 76 is generated as a minor and transient degradation product. Digestion with neutrophil elastase generated intermediates different from amyloid A 76 (Yamada et al. 1995). Despite extensive efforts, proteolysis of SAA to amyloid has not been demonstrated directly in vivo (Yakar et al. 1995).

All types of amyloid deposits contain the nonfibrillar glycoprotein amyloid P component (AP), which is identical to and derived from the normal serum amyloid P (SAP). SAP is composed of 10 noncovalently-associated identical subunits, each with a mass of 23 to 25 kD, arranged so as to face-to-face cyclic pentameric disks. In contrast to this established conception of SAP circulating as a double pentameric molecule (decamer), new data indicate that SAP circulates as a single pentamer, part of which forms complexes with C4b-binding protein (Sörensen

et al. 1995). SAP and CRP are members of the pentraxin family. SAP is a calcium-dependent, ligand-binding protein which binds to amyloid fibrils, heparan and dermatan sulfate, and to SAP itself. The peptide sequence recognised by SAP on SAP itself is a carboxyterminal peptide of residues 160-204 (Hamazaki 1994). The pentraxins CRP and SAP are known to bind to cell nuclei. In 1994 Pepys and colleagues used a system of nuclei of human cells and whole acute-phase serum as a source of proteins and confocal immunofluorescence microscopy to demonstrate that SAP binds to chromatin and to the nucleolus. Tissue amyloid P is associated with collagen and/or other matrix components in the human glomerular basement membrane and is also present on elastic fibres throughout the body. SAP is only produced by hepatocytes, and it has been shown that hepatocytes are also the single major site of SAP (and CRP) clearance catabolism in vivo (Hutchinson et al. 1994). Thus no significant catabolism of amyloid P deposited in amyloid occurs. Plasma SAP is in dynamic equilibrium with amyloid P in amyloid deposits; free exchange of SAP between the two compartments occurs during all phases of amyloid deposition and mobilization, as well as during the steady state (Hawkins 1994). The normal function of SAP is not known, nor is its role in the pathogenesis of amyloidosis.

Amyloid-enhancing factor (AEF) is a low-molecular-weight glycoprotein of 10-16 kD which associates easily with other molecules. In experimental amyloidosis, the lag phase for amyloid deposition is shortened after a single AEF injection (Gruys and Snel 1994). Extracts of amyloid have shown a high proportion of beta-pleated sheet material, and AEF has thus been suggested to act as a nucleus for amyloid fibril crystallisation.

The constant association of proteoglycans or glycosaminoglycans (GAG) with all types of amyloid suggests that they are prerequisites for amyloidogenesis. Data have accumulated indicating increased synthesis of proteoglycans at sites of amyloidogenesis prior to fibril formation (Magnus et al. 1994).

In conclusion, according to Kisilevsky and Young (1994), AA amyloidogenesis can be viewed as a sequence of the following events. First, the physiologic response to an inflammatory stimulus leads to the creation of a large pool of circulating SAA. Second, through its physiologic function in modulating HDL distribution during inflammation, SAA is preferentially distributed to macrophage-rich regions located at specific anatomic sites. These sites represent the location of amyloid formation, provided a specific set of circumstances occur. In the first set of circumstances, with long-standing inflammation, AEF may serve as a nucleus for fibril initiation and growth. In the second set, the process of fibrillogenesis involves interaction between SAA and specific basement proteins whose aberrant metabolism contributes to amyloid formation.

According to Merlini and Bellotti (2003) the conversion of the structure of the native protein into a predominantly antiparallel beta-sheet secondary structure (in which the N- and C- terminals are oriented in opposite directions) is a pathological process closely related to physiologic protein folding. The folding of a newly synthetised polypeptide occurs in a rapid sequence of conformational modifications in the cytoplasm. According to the ” folding energy landscape theory,” the process follows a funnel-like pathway in which the conformational intermediates progressively merge into a final species (Dobson and Karplus 1999). In addition, at a minimum of energy similar to that reached by the native protein, the polypeptide can acquire an alternative and relatively stable ”misfolded state” (Schultz 2000), which is prone to aggregation. Once the folding process has been completed and the native protein secreted, many proteins are in dynamic equilibrium with a partially folded conformation, and in this state retrace part of the folding pathway, ultimately forming either a native or misfolded protein.

1.5. Epidemiologia

In Finland, Laine and associates in 1955 studied the occurrence of amyloidosis in RA patients at the Rheumatism Foundation Hospital. The majority of samples were taken at orthopaedic and other

operations; in addition, routine biopsies of the skin, subcutaneous tissue, lymph nodes, muscle and other tissues were carried out, a total of 289 biopsies, and 83 (28.7%) showed morphological features and colour reactions characteristic of amyloidosis. The incidence of amyloidosis was highest in the tissues around the metatarsophalangeal joints. This may have been in part due to local stress.

The diagnosis of amyloidosis in living patients was formerly based mainly on the Congo red test, but the Congo red affinity of sera from patients with RA is low, evidently owing to their lowered serum albumin and also to the competition of Congo red with polysaccharide anions, which are increased in RA. The Congo red test has also been shown to give false-negative results in a significant proportion of histologically proven cases of amyloidosis.

Renal biopsy is a reliable method of diagnosis, but it is not without risk. In an analysis of 5000 cases, 0.7% severe complications were found, especially perirenal haematoma (Ennevaara and Oka 1964).

Data on the prevalence of amyloidosis in RA have been published based on autopsy findings. In 1986 Laakso and associates carried out a 10-year study to assess mortality and causes of death in a cohort of 1000 subjects with RA, 500 men and 500 women aged 40 years or more, together with a control population matched by age and sex. The overall mortality was significantly higher in both men and women with RA than in the controls,

this being attributable to an excess mortality from infections and cardiovascular and renal diseases. During the follow-up, 31 patients with RA (12 male,19 female) and one male control subject died of amyloidosis and 42 RA patients (19 male, 23 female) and one male control from renal diseases. The most important causes of renal deaths were chronic nephritis and renal infections.

In a clinicopathological study of 81 autopsied patients with RA in Japan, 17 (21%)

had amyloidosis (Suzuki et al. 1994). In another Japanese study, gastrointestinal fiberoscopies and biopsies were performed on 789 patients with RA on routine screening and follow-up of

amyloidosis. Seventy-seven cases (9.8%) yielded positive results for amyloidosis (Okuda et al. 1994). In contrast Okuda et al. 1994, no decline was noted in a third Japanese study (Nakano et al. 1998), where amyloidosis was present in 18% of renal biopsy specimens in patients with RA in 1979-1988 and in 19% in 1989-1996.

The incidence of amyloidosis in tissue sampling at a single site gives lower prevalence estimates than do autopsy series in the same population. It may also be the case that patients have had a severe disease and thus amyloidosis (Buxbaum 1998).

The death certificates of all 1666 RA patients who died in Finland in 1989, were investigated. Amyloidosis was regarded as an immediate cause or intervening antecedent cause of death in 64 cases (3.8%) and as a contributory cause of death in 33 (2%), corresponding to a prevalence of 5.8%. Amyloidosis had been diagnosed during life in 89 instances and was detected at autopsy in 8 (Myllykangas-Luosujärvi et al. 1999). In an earlier study based on the same material, it was estimated that 15% of the excess mortality associated with RA was due to amyloidosis (Myllykangas-Luosujärvi et al. 1995).

Survival of patients with RA and amyloidosis on dialysis has been poor. According to a surveillance study of patients entering dialysis in one Finnish dialysis unit between the years 1974 and 1987, the survival after 3 years was 37%. Among amyloidosis patients transplanted at the Finnish transplant centre over the same period it was 62% (Ylinen et al. 1992).

The prevalence of RA in Finland is 0.8%. In one Finnish series, 43% of deaths among patients with RA due to renal failure were attributed to amyloidosis (Mutru et al.1985), compared with only 20% in an English series (Symmons et al. 1998). Fewer new dialysis-requiring patients were observed at Kuopio University Hospital in the years 1989 to 1999. The number of patients with RA and amyloidosis on dialysis treatment has decreased. At the beginning of the year 2000 there was only one patient with RA and amyloidosis on dialysis in Kuopio. The decrease may be due to therapy of RA involving a shift from the administration of non-steroidal anti- inflammatory drugs (NSAIDs)

and/or glucocorticoids only to the introduction of one or several drugs in combination to modify the disease course (Kaipiainen-Seppänen et al. 2000).

Nowadays, with the use of more effective disease-modifying antirheumatic drugs (DMARDs), several authors have suggested that the incidence of amyloidosis is decreasing (Sokka et al. 1999, Myllykangas-Luosujärvi et al. 1999).

Abdominal fat amyloid deposits are not uncommon in adult Asian North Indian patients with RA. However, only one-fourth of patients with amyloid deposits have shown evidence of clinical amyloidosis. A group under Wakhlu (2003) found 30 out of 113 patients to be positive for amyloidosis by AFA (26.5%). Eight had features suggestive of clinical amyloidosis in the form of proteinuria, organomegaly, or symptomatic gastrointestinal involvement, while in 22 patients amyloidosis was subclinical (Wakhlu et al. 2003).

The most common form of amyloidosis worldwide is that which occurs secondary to chronic inflammatory disease. Amyloidosis is more commonly associated with inflammatory disease in Europe than in the USA (Buxbaum 1998). This discrepancy may be due to differences in the management of rheumatic disease, but it is possible that enviromental and genetic factors also influence the expression of amyloidosis. Most data concerning the epidemiology of amyloidosis come from autopsy studies. In several large series based on consecutive autopsies, the prevalence of amyloidosis varied from 0.5 to 0.86% in the USA (Simms et al. 1994). There may be geographic variation in autopsy rates.

Currently in the USA, fewer than 15% of individuals are autopsied. AA amyloidosis is rare in the USA, occurring in less than 1% of persons with chronic inflammatory diseases, but is more common in Europe, occurring in 5-10% of such patients.

In a Dutch series of AA amyloidosis subjects the most common underlying disease was RA (56%) (Hazenberg and van Rijswijk 1994).

In Lyon, France over the years 1977-1999, the cumulative incidence of renal amyloidosis in RA subjects observed among 6931 renal biopsies was 0.22% (15/6931). Prevalence, prognosis and the causes of death of amyloidosis patients have varied. The most common cause of death in the series in question was renal failure (Chervel et al. 2001). In a Dutch study the presenting symptoms were of renal origin in some 90% of amyloidosis patients, but renal insufficiency accounted for death in a much lower percentage (35%). Infection (16%), gastrointestinal bleeding (8%) bowel perforation (8%), and myocardial infarction (5%) were frequent causes of death both before the onset of renal failure and during haemodialysis (Janssen et al. 1986).

In 1998 Nakano and colleagues from Niigata Central Hospital, Japan, investigated renal pathology and its correlation with DMARDs in patients with RA. Renal biopsy findings in 158 Japanese RA patients with urinary abnormalities and/or renal dysfunction were analysed retrospectively between 1979 and 1996. Urological abnormality and urinary tract infection were ruled out in all patients. Light and immunofluorescence (IF) microscopy was performed in all cases. Mesangial proliferative glomerulonephritis was diagnosed in 54 patients, membranous glomerulonephritis in 49, and amyloidosis in 30. Renal dysfunction was more frequent in patients with amyloidosis (22/30) than in those without (40/128).

Recently amyloidosis was found in 35 (29%) out of 121 patients with severe RA using AFA in Lower Silesia (Wiland et al. 2004).

1.6. Diagnosis

The diagnosis and classification of amyloid requires histological evidence. No clinical or laboratory test has yet superseded histology. The sensitivity and specificity of histological diagnosis depend on the choice and adequacy of the tissue site sampled, the quantity of deposits in the sample, the quality of the equipment (a good polarisation facility is mandatory) and the awareness of the pathologist. Since AA amyloidosis is a systemic disease it may be diagnosed in

any amyloid-containing tissue, but the most common biopsy sites are subcutaneous fat, kidney, rectum and liver. When amyloid is present in small amounts it can easily be overlooked, while if present in large amounts it may be misinterpreted as elastosis in skin and breast tissue specimens or collagenous colitis in intestinal biopsies. Precise classsification of the amyloidosis type is essential, as correct treatment may delay progression of the disease. When amyloidosis is suspected, Congo red staining must be applied and the specimen viewed in polarised light in a darkened room. Following diagnosis, amyloidosis is classified either immunohistochemically using specific antibodies directed against defined fibril proteins (Röcken et al. 1996) or biochemically by extracting the fibril proteins from native or formalin-fixed amyloidotic tissue and submitting the protein to amino acid sequencing. Finally, the histopathological diagnosis and classification of amyloidosis should be correlated with the clinical information, since the data provided may be misleading. For instance, a patient with plasmacytoma (which may be associated with amyloid light-chain (AL) amyloidosis) may suffer from RA (which may be associated with AA amyloidosis), of which the pathologist
may be unaware.

Over the years 1976-1992 Helin and colleagues investigated a total of 1801 renal biopsies at Tampere University Hospital. In the retrospective study of renal biopsy specimens from 110 RA patients in whom the clinical renal disease was probably due to RA itself or to antirheumatic therapy, the most common histopathologic finding was mesangial glomerulonephritis (n=40), followed by amyloidosis (n=33). Amyloidosis was the most
common finding in patients with nephrotic syndrome. The renal morphological lesion in RA patients with isolated proteinuria and in those with haematuria cannot be accurately predicted on the basis of clinical symptoms and signs. Biopsy is thus mandatory for differential diagnosis (Helin et al. 1995).

1.7. Clinical features

TNF has a key role in the inflammatory cascade resulting in erosive arthritis in RA.

Circulating TNF and soluble sTNF R1 levels are significantly raised in RA patients with amyloidosis as compared with those without amyloidosis, and raised TNF levels may be implicated in the pathogenesis of certain disease manifestations such as anaemia of chronic disease, muscle wasting, cachexia and nephropathy with proteinuria but no creatinine elevation (Maury et al. 1991, 2003).

Proteinuria is usually the first clinical presentation of systemic amyloidosis, and the severity of albuminuria correlates with the patient´s prognosis (Joss et al. 2000). Rare extensive involvement of the heart leads to cardiac failure. Adrenocortical insufficiency due to amyloid deposition in the adrenal glands has been described but is rare, and is usually associated with extensive renal amyloidosis. Amyloid deposits may be found in the gastrointestinal tract, either in the bowel wall or in the blood vessels (Lee et al. 1994). Bleeding from amyloid lesions within the gut may be exacerbated by the non-compliant nature of amyloidotic vessels. Extensive infiltration may result in malabsorption, intestinal obstruction or pseudo-obstruction.

Falck and associates (1983a) described 9 patients with amyloidosis with little or no proteinuria; predominantly vascular amyloid deposition was found in renal biopsy specimens from all 9 patients.

Hepatomegaly has been described in some 20% of patients with amyloidosis (Janssen et al. 1986). Amyloidosis causes liver enlargement rather than functional disturbances. In 1988 Tiitinen and associates reported a case of a 68-year-old woman who had had RA for 12 years. Her liver extended to her navel. Biopsies from liver and AFA revealed amyloidosis, and immunohistochemical examination of the liver cutlet stained strongly with anti-SAA antiserum. Her condition was satisfactory.

Splenomegaly is also a feature of amyloidosis. In one series of 91 patients with RA and amyloidosis, signs of cardiomyopathy, neuropathy and goitre with or without hypothyroidism were rare, enteropathy was seen in 24% and hepatomegaly in 18% (Hazenberg and van Rijswijk 1994). It is of interest that although RA occurs twice more often in females, the incidence of amyloidosis is equally distributed between the sexes, suggesting an increased susceptibility in males (Cohen 1968). In 1987 Myllykangas and Tiitinen concluded that amyloidosis is not only more prevalent in male patients, but also seems to be more rapidly progressive in men with chronic inflammatory arthritis (Myllykangas et al. 1987).

In 1993 Korpela reported that the prevalence of renal amyloidosis in 1042 patients with RA was 4.2% in males and 2.3% in females. In RA which had lasted for 20 years or more renal amyloidosis was found in 8.5% of men and in 4.5% of women.

1.8. Progression

The importance of SAA in the progression of renal failure was studied over three years in 28 patients with amyloidosis predominantly attributable to RA. Creatinine clearance, the quantity of protein in urine, and SAA and CRP concentrations were determined regularly. Linear regression analysis showed a close correlation between the decrease in creatinine clearance each year and both SAA concentrations (20 patients: $r=0.82$, $P<0.001$) and CRP concentrations (28 patients: $r=0.80$, $P<0.001$). The correlation between SAA and CRP concentrations was also significant (317 parallel measurements: $r=0.81$, $P < 0.001$). These findings suggest that monitoring SAA or CRP concentrations is of value in assessing the prognosis in amyloidosis and that therapeutic intervention designed to lower SAA concentrations may reduce the formation of amyloid (Falck et al. 1983b).

In 2001 Gillmore and colleagues studied retrospectively for 12-117 months 80 patients with amyloidosis, in whom the serum SAA concentration was measured monthly. Visceral amyloid

deposits were assessed annually by serum amyloid P component scintigraphy. Underlying inflammatory diseases were treated as vigorously as possible. Estimated survival at 10 years was 90% in patients whose median SAA was under 10 mg/l, and 40% among those whose median SAA exceeded this value (P=0.0009).

1.9. Prognosis

During the 1970s a number of studies demonstrated the grave prognosis of amyloidosis. The natural progression of the condition has been found to vary, with an overall mortality of 50% in 5 years and 90% in 10 years (Wegelius et al. 1980). The main cause of death is renal failure. The best prognosis of amyloidosis is in JRA, the poorest in men with seropositive RA (Korpela 1993).

The proportional acceptance rate of uremia patients for renal replacement therapy has been over 10% in Finland, the highest in Europe. From 1973 to 1981 45 patients were accepted , and their actual 3- and 5-year graft survival rates were 41% and 32%. These patients had conventional immunosuppression. In 1981 cyclosporin was introduced for renal graft therapy. Actual graft survival rates were 68% and 33%, respectively. All manner of complications such as rejection of the renal allograft, the side-effects of the medication, cardiovascular problems and infections in patients with amyloidosis were concentrated in the first three months after transplantation (Kaija Salmela, personal communication 2003), the first year after tranplantation being the most troublesome.

Recurrence of amyloidosis in the renal allograft has been documented in several case reports, but the exact frequency is as yet unknown. In one study 3-year survival (51%) was inferior to that of controls, but the 3-year graft survival rate was nearly the same in amyloidosis patients (38%) as in controls (45%) (Pasternack et al. 1986).

1.10. Treatment

Twenty-two patients with amyloidosis secondary to RA were randomised and followed prospectively in order to determine whether treatment with cytotoxic drugs (podophyllotoxin derivatives, chlorambucil, azathioprine and cyclophosphamide) could postpone the development of end-stage renal failure. The diagnosis of amyloidosis was based on albuminuria, microscopic haematuria and rectal and/or AFA and/or renal biopsies. Renal function was followed by repeated measurements of the glomerular filtration rate (GFR). Urinary albumin and serum creatinine were found to be unreliable as predictors of renal function. GFR declined more rapidly in the patient group receiving only symptomatic drugs and no cytotoxic drugs . After an initial decline, the GFR in the cytotoxic drug treatment group, mean treatment quotient 79%, levelled off and remained constant for a considerable time. The mean observation times were 46 and 54 months. The difference in favour of cytotoxic drugs was significant ($P<0.04$) (Ahlmen et al. 1987).

Berglund and associates assessed renal outcome in 16 consecutive patients with RA and amyloidosis treated with alkylating agents and reviewed management principles over 21 years. Renal function was assessed by serum creatinine and the albumin/creatinine clearance ratio, and arthritic activity by joint score and CRP. In the event of renal deterioration, cyclophosphamide (CYC), or since 1975 chlorambucil, was given until stable remission of arthritis was obtained. By 1992 the median survival of renal function was 11 years (range 4-21). The results indicate that the survival of renal function may be substantially prolonged (compared to no treatment) when cyclophosphamide, or preferably chlorambucil, is appropriately administered for active arthritis, and lifelong, continuous monitoring maintained (Berglund et al. 1993).

The efficacy of cyclophosphamide combined with prednisolone has been studied in amyloidosis patients who had the SAA 1.3 genotype, which is a risk factor for amyloidosis in Japanese RA patients. Fifteen RA patients with amyloidosis who were SAA 1.3 homo-or heterozygotes were treated with the combination, and CRP, RF, ESR, albumin and creatinine levels improved with the

treatment ($P<0.05$) (Nakamura et al. 2003). SAA 1.3 homozygotes constitute about 10% of Japanese RA patients, and SAA 1.3 heterozygotes about 20%.

The current treatment strategy in patients with amyloidosis is to reduce SAA values to under 10 mg/l (Gillmore et al. 2001). If RA could be treated as successfully as infectious diseases, one might expect a similar decline in the development of amyloidosis (Hazenberg and van Rijswijk 2000). There are also findings suggesting that the disappearance of amyloidosis may reflect the use of DMARDs. RA patients treated with CHQ have a greater risk of developing amyloidosis compared to patients treated with other drugs such as gold salts, sulphasalazine or cytotoxic drugs (Prokaeva et al. 1995). Between 1996 and 2001 in Wroclaw 121 RA patients were studied. Significantly fewer patients with amyloidosis had previously taken methotrexate than those without (25% vs 45%; $P< 0.01$) (Wiland et al. 2004). MTX may lead to a decrease in or even complete resolution of proteinuria in RA patients with amyloidosis (Fiter et al. 1995).

Other concomitant diseases such as hypertension or infections should be effectively treated. Cases with hypertension and/or proteinuria are treated with angiotensin-converting-enzyme (ACE) inhibitors. Loop diuretics are the main therapeutic agents for the treatment of fluid overload, but digoxin and calcium-channel blockers should be avoided. The mechanism of toxicity of the latter drugs appears to be related to their ability to bind to amyloid, this increasing their concentration in the myocardium (Buxbaum 1998).

If renal failure becomes progressively worse, and complete renal insufficiency is imminent patients are recommended to limit their sodium intake, and in severe proteinuria (more than 8g/24h) to use protein 0.8g per kg body weight daily (Ruggenenti et al. 1997).

2. AIM OF THE STUDY

The present study was started in 1987, when death from renal failure in patients with RA, proteinuria and amyloidosis could occur within a few years unless the inflammatory process was controlled. In rheumatology risk factors for amyloidosis were crucial.

As all patients do not develop amyloidosis, the present study sought further for differences between RA patients with and without amyloidosis. The specific aims of this series were to establish:

1. What is the utility of the present method of AFA in the diagnosis of amyloidosis?
2. What is the 15-year incidence and what are the early risk factors for amyloidosis in patients with RA?
3. What are the differences in HLA types between RA patients with and without amyloidosis in Finland and in Poland?
4. What is the role of apolipoprotein E phenotypes in RA patients with and without amyloidosis?
5. What is the role of circulating interleukin-18 in RA patients with and without amyloidosis?
6. How effective was treatment with DMARDs in RA patients with amyloidosis in Finland and Poland before 1991?
7. Does serum CRP reflect the activity of disease in patients with amyloidosis and proteinuria?
8. Has the incidence of amyloidosis decreased in patients with inflammatory joint disease in Finland?

3. PATIENTS AND METHODS

3.1. The appearance of amyloidosis was studied during two periods using different methods of biopsy. During the first period biopsy was performed traditionally by rectal or gingival biopsy, and during the second by the AFA method. AFA specimens were obtained by aspiration of subcutaneous abdominal adipose tissue, using an adaptor with a 10-ml syringe connected to a blood needle (1.2 mm inner diameter), from the sub-or paraumbilical area of the abdomen. Small samples of adipose tissue were smeared onto two precleaned glass slides and allowed to air dry overnight before staining with Congo red. The material was examined using polarised light microscopy. The numbers of new amyloidosis cases were roughly compared to all RA patients at the Rheumatism Foundation Hospital throughout both periods. Mortality among amyloidosis patients was noted.

3.2. Between 1973 and 1975 a total of 441 patients with recent arthritis (duration of disease not more than 6 months) were studied at the Rheumatism Foundation Hospital (Kaarela 1985). A follow-up for patients with RA was undertaken at 1 year from entry and was repeated at 3, 8 and 15 years from entry. At the 15-year check-up 74 erosive and seropositive RA patients attended, and AFA was performed. The death certificates of 24 deceased patients and patient documents of 4 non-participating patients were examined. Five had died of amyloidosis. These 5 and 6 biopsy-proven amyloidosis patients formed the amyloidosis group, and the 68 yielding negative biopsy and 13 of the deceased patients formed the control group.

To study early prognostic signs of amyloidosis, age, tests for HB, ESR, CRP, serum orosomucoid, serum globulin, immunoglobulins and the Waaler-Rose test were carried out at the Rheumatism Foundation Hospital and the Laboratory of Clinical Immunology, Helsinki.

Morning stiffness lasting at least 1 hour , number of swollen joints, erosiveness and the

first DMARD during initial hospitalisation were registered, together with HLA-B27. The same laboratory findings were registered at the 3-year examination. The amyloidosis and control groups were analysed by Student´s unpaired t-test, Mann-Whitney test and Fisher´s exact test.

3.3. HLA typing was carried out on 87 Finnish RA and 60 RA and amyloidosis patients from the Rheumatism Foundation Hospital, and on 77 Polish RA and 23 RA and amyloidosis patients from the Institute of Rheumatology, Warsaw, Poland. In all patients in Finland amyloidosis was confirmed by AFA, and by rectal biopsy in 15 and by gingival biopsy in 8 in Poland. HLA-A, -B, -C and –DR antigens were determined in patients and healthy donors at the Finnish Red Cross Blood Transfusion Service, Helsinki, and at the HLA Laboratory of the Institute of Rheumatology, Warsaw.

Fisher´s exact test was used to compare the HLA frequencies. Differences was taken to be significant if the corrected P value was <0.01.

3.4-5. A total of 110 adult patients, 91 women and 19 men, median age 58 years (range 35-79 years) with seropositive and erosive RA fulfilled the 1987 revised criteria of the American College of Rheumatology (Arnett et al. 1988). All patients were evaluated at the Rheumatism Foundation Hospital. On the basis of Congo red staining and greenish birefringence in polarisation microscopy in tissue biopsies (AFA 50, rectum 2, gingival-tongue 2 and kidney 1) amyloidosis was diagnosed in 55 patients. AFA was negative in the remaining 55 RA patients.

Apo E phenotypes were determined by isoelectric focusing and immunoblotting at the National Public Health Institute, Helsinki, Finland.

For statistical analysis the chi-square test and unpaired, two-tailed Student´s t-test were used. P<0.05 was considered significant.

Plasma IL-18 was measured by sandwich enzyme-linked immunosorbent assay using two different anti-IL-18 monoclonal antibodies (Human IL-18 ELISA MBL, Nagoya, Japan). The sensitivity of the assay is 12.5 ng/l, and the intra-assay and inter-assay coefficients of variation are <11%.

CRP was measured by a solid phase immunosorbent assay based on the sandwich principle (IBL Immuno-Biological Laboratories, Germany) or by immunoturbimetry (Boehringer Mannheim). For statistical analysis two-tailed Student`s t-test for unpaired data, chi-square test and linear regression analysis were used. Values were expressed as mean ±SEM or as median and ranges.

3.6. In Finland and Poland 69 patients with RA and amyloidosis were treated with AZA, COL or MTX. The Polish AZA group comprised 16, and the Polish COL group 15 patients. Amyloidosis was confirmed by rectal biopsy in 19 and by gingival biopsy in 12 patients. AZA was initiated at 0-22 months (mean 8, SD 8), and COL at 0-28 months (mean 10, SD 9) after the diagnosis of amyloidosis.

The Finnish AZA group comprised 24 patients and the Finnish MTX group 14. Amyloidosis was confirmed by AFA. Finnish patients (but not Polish) with serum creatinine levels of more than 255 micromol/l were excluded. AZA was initiated at 0-26 months (mean 5, SD 7) and MTX at 0-13 months (mean 2, SD 4) after the diagnosis of amyloidosis. For statistical analysis the paired t-test (two-tailed) was used.

3.7. The study group consisted of 19 patients with amyloidosis and proteinuria (14 with RA and 5 with JCA). ESR, CRP concentration and urinary protein excretion were evaluated using routine laboratory methods. S-CRP was measured with both immunoturbidimetric and radioimmunoassays. The concentration of urinary CRP was measured with a double-antibody radioimmunoassay. In brief, CRP in urine competes with a fixed amount of 125 I labelled CRP for the binding sites of specific rabbit antibodies. The bound CRP is precipitated with sepharose-bound antibodies to rabbit IgG, then centrifuged and the radioactivity of the pellets is counted. The detection limit of the assay is 0.005 mg/l.

3.8. A total of 4508 biopsies were performed at the Rheumatism Foundation Hospital to reveal amyloidosis in patients with RA, JCA, psoriatic arthritis or spondyloarthropathy between 1987 and

1997. Few rectal biopsies were performed early in the decade, but AFA was the routine method and tongue-gingival biopsy an alternative. All samples were examined by the same pathologist.

4. RESULTS

4.1 Modern trends in the diagnosis of amyloidosis (I)

During the first period, from September 1979 to September 1985, 130 RA patients were diagnosed as having amyloidosis. At the time of biopsy 90% of the patients had proteinuria. During the second period, from September 1985 to September 1987, amyloidosis was diagnosed in 82 patients. Now 50% of patients had proteinuria. At the end of follow-up, 62 (48%) of the RA patients from the first period had died, 45 of renal failure.

4.2. Amyloidosis - incidence and early risk factors in patients with RA (II)

At the 3-year follow-up of this inception cohort study, 102 patients had RA. At the 15-year check-up 74 living patients were investigated. The incidence of amyloidosis in these patients was 8.1% (6/74). Between the 3-year and the 8-year studies 6 patients with RA had died, but none of amyloidosis. After the 8-year check-up in 1987, 18 had died, 5 of amyloidosis. Assuming that 19 of the deceased patients did not have amyloidosis the 15-year incidence of amyloidosis in RA was 10.8% (11/102) or 13.9% (11/79) if all 23 patients without biopsy are excluded.

At onset, of the 14 risk factors investigated only high serum orosomucoid distinguished the 11 amyloidosis patients from the 81 without amyloidosis. However, at the 3-year check-up patients with subsequent amyloidosis more often experienced morning stiffness (P=0.02), higher ESR (P<0.007), more often positive CRP (P=0.006) and higher serum orosomucoid (P=0.04). Additionally, the amyloidosis patients then had a mean of 11 swollen joints compared with the 5 among the controls (P=0.05, not significant). These results woud indicate that active RA during the first 3 years is a clear risk factor for amyloidosis. Early HCQ treatment was significantly (P=0.04) less effective than GSTM,

emphasising the importance of proper treatment of RA at its onset.

4.3. HLA typing and seropositivity in Finnish and in Polish patients with RA and amyloidosis (III)

As it has previously been suggested that the Finnish population, with a high HLA-B27 frequency, is genetically susceptible to amyloidosis, Finnish and Polish RA patients were compared with respect to HLA types. The RA patients in Finland and Poland were comparable in sex and age, but age at the diagnosis of amyloidosis was significantly higher among the Finnish patients. All the Finnish patients with amyloidosis had been at least once RF positive, the corresponding figure for the Polish patients being 70%. As to the HLA frequencies, there were no significant differences between the RA patients with and those without amyloidosis in Finland or in Poland. As expected, HLA-DR4 was significantly ($P<0.001$) more frequent in all four patient groups than in the blood donors. In Finland, the RA patients with amyloidosis had a lower frequency of HLA-DR2 than the blood donors ($P=0.0108$), and RA patients without amyloidosis evinced a significantly lower frequency of HLA-DR7 ($P<0.001$).

4.5. Apolipoprotein E phenotypes in RA with or without amyloidosis (IV)

The Apo E3/3 phenotype was significantly more common (71%) in the RA patients without amyloidosis than in the amyloidosis group (49%, $P<0.05$) or the Finnish control population (47%, $P<0.01$). The Apo E3/4 phenotype tended to be more common in the RA and amyloidosis patient group (40%) than in the non-amyloidosis group (26%), but the difference was not statistically significant.

RA patients without amyloidosis had a significantly higher frequency of Apo E3 (0.86) than RA and amyloidosis patients (0.73, $P<0.05$) or control subjects (0.73, $P<0.01$). The Apo E4 frequency was lower in the non-amyloidosis RA patients (0.13) than in controls (0.23, $P<0.05$) or RA and amyloidosis patients (0.23, NS).

4.5. Raised circulating interleukin -18 levels in AA amyloidosis (V)

The circulating levels of IL-18 were significantly elevated in RA patients as compared with control subjects. Those RA patients who had amyloidosis had a significantly higher mean circulating level of IL-18 than those without amyloidosis (418.1 ± 32.1 ng/l versus 317.0± 21.3 ng/l, $P<0.02$). This difference was not due to differences in inflammatory activity, nor was it related to renal function.

4.6. Treatment of amyloidosis with AZA, COL or MTX in patients with RA and amyloidosis in Finland and Poland (VI)

In the AZA group in Finland (total number 24) 15 patients had proteinuria of more than 0.1 g/l before and 13 after the treatment, and the serum creatinine concentration exceeded 100 micromol/l in 8 patients before and in 10 after treatment.

In the AZA group in Poland (16 patients) serum albumin levels decreased, and serum creatinine rose clearly. All Polish patients had proteinuria (mean 2 g/l before and mean 6 g/l after AZA treatment). Serum creatinine was over 100 micromol/l in 4 patients before and in 12 after treatment.

In the COL group in Poland (15 patients) serum albumin levels decreased. All these patients had mean proteinuria 2.2 g/l (SD 2) before and mean 2.2 g/l (SD 3) after treatment. The serum creatinine concentration exceeded 100 micromol/l in 7 patients before and in 10 after COL treatment.

In the MTX group in Finland (14 patients) the means of ESR and HB were significantly improved after treatment, and blood platelets and serum gammaglobulin concentration decreased. Six patients in this group had proteinuria before and 4 after treatment, and the serum creatinine concentration exceeded 100 micromol/l in 3 patients before and in 2 after treatment.

4.7. Serum CRP is rarely lost into urine in patients with amyloidosis and proteinuria (VII)

The activity of disease in the 19 amyloidosis patients was assessed by ESR and CRP. The mean ESR was 83 mm/h and CRP only 24 mg/l. Proteinuria ranged from 0.4 to 12.3 g/24h. Thus, as Table 1 in VII shows, it was possible to establish whether CRP was lost into urine. Four out of nine patients with heavy proteinuria showed measurable amounts of urinary CRP. The CRP level did not correlate with the amount of urinary CRP when the level of proteinuria was less than 8 g/24h.

4.8. Amyloidosis has decreased in patients with inflammatory joint disease in Finland (VIII)

A total of 4508 biopsies had been performed at the Rheumatism Foundation Hospital during 1987-1997. The annual number of new amyloidosis patients decreased from 68 in 1988 to 3 in 1997. On the other hand, the annual hospitalisations doubled during the same period, from 3600 to 7011 patients.

5. DISCUSSION

5.1. Prognosis of amyloidosis patients

The prevalence of amyloidosis in RA in different studies has been 5-10% (Dhillon et al. 1989). The prognosis of RA is poorer than in any other common rheumatic disease, and is worst with amyloidosis and renal failure, which may be sudden when there is renal vein thrombosis. Drug treatment has no effect on these late stages, but expensive dialysis and renal transplantion are necessary. These facts formed the basis for the present series, where the problem of amyloidosis was taken into account from the onset of RA, and they are discussed in the following sections.

5.2 . Modern trends in the diagnosis of amyloidosis

In paper I the AFA method introduced at the time in the Rheumatism Foundation Hospital is described. This simple new method allowed repeated biopsies and milder indications. The result was that the incidence of amyloidosis doubled, and the condition was detected at an earlier stage. In the clinical situation the specificity of AFA is rarely a problem. A patient with erosive polyarthritis, ESR over 50 mm/h and CRP over 40 mg/l should be considered for MTX treatment, which was not self-evident in 1987 without positive biopsy.

A longer study with much less strict indications for biopsy was performed in Spain, where Gomez-Casanovas and collegues over a 14-year-period (1983-1997) systematically perfomed an AFA test on all their 313 RA patients with more than five year disease duration.

The prevalence of amyloidosis was 19.5%. Only 26 (42.6%) of these AFA-positive patients had proteinuria or renal insufficiency or both. A renal biopsy was obtained in 11 cases, confirming histological amyloidosis in 10 of them. Amyloid deposits seem to be silent in this subclinical amyloidosis, even after a follow-up of several years (Gomez-Casanovas et

al. 1998). Thus an AFA-positive test in selected patients with RA does not necessarily imply clinical systemic amyloidosis and poor prognosis.

5.3. Amyloidosis - incidence and early risk factors in patients with RA

Apart from the present study, there is no long-term inception cohort study dealing with amyloidosis in RA. The follow-up commenced in 1973-75. At the 15-year check-up in 1989 AFA was performed on all patients, thereafter only on subjects with proteinuria or high serum creatinine. Death certificates were checked. At the 15-year check-up 6 of the initial 102 patients had amyloidosis by AFA, and 5 of the 24 deceased patients had had amyloidosis. Thus the 15-year incidence of amyloidosis in erosive RA was at least 11% (11/102). At the 20-year follow-up in 1995, cumulatively 14 RA patients had had amyloidosis. The 20-year check-up of these patients confirmed that patients with amyloidosis had had worse disease: Larsen score of 0-100 was 57, whereas in those without amyloidosis it was 40 (P=0.026) (Jäntti et al. 2002). Up to 2002, cumulatively, 16% of patients had developed amyloidosis (Kaarela et al. 2002).

In paper II the duration of disease was less than six months at entry. In 1985 Kaarela published entry variables and the outcome of the same RA patient population. As the number of swollen joints, Waaler-Rose test results, morning stiffness, ESR, age, serum globulins, serum orosomucoid and CRP in this order correlated ($0.49 > r > 0.23$) with poor prognosis for RA, this cohort were chosen for a study of the early prognostic factors in amyloidosis. The amyloidosis patients seemed initially to have a slightly more active disease while after three years the values for morning stiffness, ESR, CRP and orosomucoid were significantly and the number of swollen joints nearly significantly higher than in patients without amyloidosis over 15 years. In other words, continuous long-lasting inflammatory activity is the principal risk factor for the development of amyloidosis. The validity of single variables in the measurement of activity in RA has been studied, and the best single indicator proved to be the number of swollen joints, while

CRP and ESR had less discriminating power (van der Heijde et al. 1992).

In paper II GSTM, HCQ and prednisolone were the drugs used in early RA in Finland. Sulphasalazine was used very rarely, and penicillamine treatment was not introduced until 1975. In the present study HCQ was started in 64% of patients with later amyloidosis compared with 28% of those without. The treatment policy for RA at the Rheumatism Foundation Hospital since the 1950s had been active GSTM treatment (Lehtinen and Isomäki 1991), so that patients with active disease were presumably treated less often with GSTM than was usually the case. HCQ monotherapy has had modest effects on erosive RA, and has been shown to cut down the number of erosions significantly less than sulphasalazine (van der Heijde et al. 1989).

5.4. HLA typing and seropositivity in Finnish and Polish patients with RA and amyloidosis

The high prevalence of amyloidosis in Finland would suggest that the geographically isolated Finnish population has probably a genetic susceptibility to amyloidosis in association with RA. A comparison was therefore undertaken of the distribution of HLA antigens in patients with amyloidosis associated with RA in two different populations, one from Finland and one from Poland. The prevalence of HLA-B27 in the Finnish population is 15%. Similar high figures have been reported from Northern Scandinavia (Rantapää Dahlqvist 1986) and Estonia (R.Birkenfeld, personal commmunication 1991). The 5% frequency observed in Poland is comparable to other European figures. Our results confirmed the increased frequency of 30% of HLA-B27 previously described for Finnish RF-positive RA patients (Nissilä et al. 1988).

In 1988 Maury and associates reported a high frequency of HLA-DR4 and a low frequency of HLA-DR2 content in Finnish patients with RA and amyloidosis. In the present study the figures were similar (Maury et al. 1988).

All these amyloidosis patients from Finland and all the 300 amyloidosis patients with RA

diagnosed by the author between 1985-1991 were RF-positive. This high figure is explained not so much by exclusion of seronegative spondylarthritis, as rather by the long-lasting follow-up - often for decades - of RA patients at the Rheumatism Foundation Hospital. Only 31% of Waaler-Rose test-positive patients are continuously seropositive during the first eight years (Kaarela 1985).

5.5. Apolipoprotein E phenotypes in RA with or without amyloidosis

Data on the prevalence of Apo E4 isoprotein in amyloidosis have been contradictory. Factors such as the selection of patients, the heterogeneity of the underlying disease and/or lack of adequate disease controls, and ethnic differences may have influenced results. The frequency of the Apo epsilon4 allele in fact shows great variation in different populations, ranging from 0.07 to 0.30.

Notwithstanding this, the fact that the frequency of the Apo epsilon4 allele is significantly decreased in RA patients without amyloidosis when compared with Finnish control subjects may suggest that the presence of Apo E4 isoprotein in a patient with RA could increase the relative risk of developing amyloidosis.

5.6. Raised circulating interleukin-18 levels in AA amyloidosis

The results of this study show for the first time that amyloidosis patients have increased levels of circulating IL-18, levels being significantly higher in RA patients with amyloidosis than in patients without. Since the amyloidosis and non-amyloidosis patient groups were matched with respect to age, sex, duration of RA and seropositivity, these factors are unlikely to have affected the results. The type of medication was also very similar in both groups. The higher IL-18 concentration in the amyloidosis group was probably not a result of differences in inflammatory activity, since there were no significant differences in CRP levels between amyloidosis and non-amyloidosis patients, nor a result of differences in renal function, since IL-18 levels did not correlate with serum

creatinine levels.

5.7. Treatment of amyloidosis with AZA, COL or MTX in patients with RA in Finland and Poland

In the present study 69 patients were treated with AZA or COL or MTX. The groups were small, as often even in international amyloidosis studies, and the observation times were relatively short. However, the follow-up revealed a significant worsening in renal function except in those receiving MTX.

In Finland the mean laboratory parameters in the AZA group were worse than in subjects treated with MTX, as patients with a glomerular filtration rate less than 0.8 ml/s/ 1.73m^2 were not accepted in the MTX group. The early diagnosis of amyloidosis in the latter patients was based on the AFA method. In Poland all patients had proteinuria or nephrotic syndrome. Also in the Finnish AZA group 15/24 had proteinuria. Treatment at a later stage of amyloidosis may partly explain the sparse results in the AZA group.

In amyloidosis associated with the recessively inherited familial Mediterranean fever, COL is capable of preventing febrile attacks as well as the development of amyloidosis, and is also effective in established amyloidosis before organ failure is evident. COL appeared to be more effective than AZA in this particular inflammatory disease (Husby 1998).

Paper VI clearly shows the difficult clinical situation which arises if conventional treatment of RA is started at a later stage of amyloidosis. Prevention of amyloidosis should be initiated at the onset of RA. Even the history of gold treatment confirms this (Lehtinen and Isomäki 1991).

Subsequently, gold treatment was gradually replaced by MTX. Since 1985 amyloidosis has been treated with MTX at the Rheumatism Foudation Hospital. In the earliest abstract paper MTX treatment improved the means of ESR, CRP and HB significantly and renal function did not deteriorate (Tiitinen and Kaarela 1990).

A 3-year, open randomised clical study was conducted to evaluate the therapeutic efficacy, tolerability and safety of a podophyllotoxin derivate Reumacon (300mg/day) versus AZA (2.0-2.5 mg /kg /day) in the treatment of 60 patients (49 females, 11 males) with RA and amyloidosis. Patients with severe renal disease (serum creatinine > 150 micromol/l or proteinuria > 3.0 g/24h) were excluded. During the treatment, the mean creatinine clearance (ml/s/1.73 m^2) decreased from 1.24 to 1.17 in the Reumacon group (NS) and from 1.44 to 1.09 in AZA group (P=0.03). The mean degree of proteinuria (g/24h) increased from 0.39 to 1.01 inthe Reumacon group (NS) and from 0.12 to 0.20 in the AZA group (NS) (Korpela et al. 1998).

In 2001 Gillmore and associates studied retrospectively for 12-117 months 80 patients with systemic amyloidosis in whom the serum SAA concentration was measured monthly and visceral amyloid deposits were assessed annually by serum amyloid P component scintigraphy. Underlying inflammatory diseases were treated as vigorously as possible.

Amyloid deposits regressed in 25 out of 42 patients whose median SAA values were within the reference range (<10 mg/l) throughout follow-up. The outcome varied substantially among patients whose median SAA concentration exceeded 10 mg/l, but the amyloid load increased and organ function deteriorated in most of those whose SAA was persistently above 50 mg/l. Estimated survival at 10 years was 90% in patients whose median SAA was less than 10 mg/l, and 40% among those whose median SAA exceeded this value (P=0.0009) (Gillmore et al. 20001).

A multicentre trial using a new anti-amyloid drug, Fibrillex (1.3-propanedisulfonate) is under way for the treatment of amyloidosis. Fibrillex has been designed to interfere with the interaction of amyloidosis protein with glycosaminoglycans in tissues and, thus, to prevent fibril formation and deposition.

5.8. Serum CRP is rarely lost into urine in patients with amyloidosis and proteinuria

The current treatment strategy in patients with amyloidosis is to reduce SAA values to below 10 mg/l (Gillmore et al. 2001). However, SAA investigations were not included in our study, and are not routinely used in Finland even today. ESR is not a reliable measure of disease activity in such RA patients who have anaemia and who have hypoalbuminaemia arising from proteinuria. As CRP usually correlates with SAA , it is used in clinical practice to assess the efficacy of treatment. It was previously assumed at the Rheumatism Foundation Hospital that S-CRP was lost into urine in proteinuric patients and the S-CRP value is thus reduced. To test this hypothesis the present study was undertaken. The finding was that S-CRP is lost into urine in only negligible amounts in patients with amyloidosis and proteinuria when urinary protein loss is less than 8 g/24h; the S-CRP can thus be used in the evaluation of activity in amyloidosis. When proteinuria exceeds 8 g/24h; the S-CRP level may be reduced by excretion.

5.9. Amyloidosis has decreased in patients with inflammatory joint disease in Finland

In Finland 15% of excess deaths of patients with RA were due to amyloidosis in 1989 (Myllykangas-Luosujärvi et al. 1999). As the prevalence of amyloidosis constituted 5.8% among the patients who died, it can be calculated that the prevalence of amyloidosis in all Finnish RA patients (estimated figure 30000) was about 1740.

Paper I reported that the yearly incidence of amyloidosis at the Rheumatism Foundation Hospital rose to 41 when AFA came into use. Thereafter, as paper VIII demonstrates, the annual number of positive cases decreased from 68 to 3. The same trend was later confirmed at Kuopio University Hospital, where the need for dialysis due to amyloidosis was reduced (Kaipiainen-Seppänen et al. 2000). In Finland today, there are no reports of children with JCA and amyloidosis (Savolainen 1998, personal communication 2005).

It is generally accepted that amyloidosis caused by tuberculosis has disappeared from

Western countries as a result of proper antimicrobial treatment. Myllykangas-Luosujärvi and co-workers, Sokka and co-workers and Kaipiainen-Seppänen and co-workers have suggested that the diminished figures for amyloidosis has been in Finland a result of more intensive drug treatment of rheumatic diseases (Hazenberg and van Rijswijk 2000) .

In Finland, the nationwide statistics of the Social Insurance Institution allow survey of the yearly change in the use of DMARDs. The use of MTX rose from 6800 patients in 1995 to 19628 patients in 2003 (Klaukka and Kaarela 2003). As the use of other cytotoxic drugs has decreased, MTX and combination treatments with MTX seem to be the reason for the reduction in the incidence of amyloidosis in patients with chronic inflammatory joint disease.

6. SUMMARY AND CONCLUSIONS

1. As AFA is an easy and simple procedure even for outpatients, its use can double the incidence of amyloidosis.

2. During 1973-75 a total of 441 patients with recent arthritis (duration of disease not more than 6 months) were studied at the Rheumatism Foudation Hospital. This prospective 15-year study confirms the clinical impression that patients with active disease run a greater risk of developing amyloidosis. As the amyloidosis series consisted of only 11 patients, no far-reaching conclusions could be drawn as to prognostic aspects. The 15-year incidence of amyloidosis was 11% (11/102).

3. All the Finnish patients with amyloidosis in this series had been at least once RF-positive, the corresponding figure for the Polish patients being 70%. As to HLA frequencies there were no significant differences between the RA patients with and those without amyloidosis in Finland or in Poland.

4. The prevalence of the Apo E4 isotype was not increased in patients with RA complicated by amyloidosis when compared with Finnish control subjects. However, the fact that the frequency of the Apo epsilon4 allele is significantly decreased in RA patients without amyloidosis when compared with Finnish control subjects may nonetheless suggest that the presence of the Apo E4 isoprotein in a patient with RA might imply a relative risk of developing amyloidosis.

5. RA is associated with increased levels of plasma IL-18, levels being significantly higher in patients who have developed amyloidosis. The increased level in amyloidosis patients is not a consequence of renal insufficiency, nor is it related to inflammatory activity.

6. In an open prospective study in Finland and in Poland, 69 patients with RA and

amyloidosis were treated with AZA, COL or MTX. In the AZA and COL groups renal insufficiency became worse, while in the MTX group ESR, CRP and HB seemed to improve, and renal function did not deteriorate.

7. S-CRP is lost into urine in negligible amounts in patients with amyloidosis. Only when urinary protein loss is more than 8 g/24h are significant quantities of CRP detected in urine.

8. It is suggested that a change in medication towards more frequent use of MTX may be the reason for the decreased incidence of amyloidosis in Finland.

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