MARJA PERTOVAARA

Clinical Features and Outcome of Primary Sjögren´s Syndrome

■

ACADEMIC DISSERTATION

To be presented, with the permission of
the Faculty of Medicine of the University of Tampere,
for public discussion in the small auditorium of Building B,
Medical School of the University of Tampere,
Medisiinarinkatu 3, Tampere, on August 24th, 2001, at 12 o'clock.

Acta Universitatis Tamperensis 828
University of Tampere
Tampere 2001

ACADEMIC DISSERTATION
University of Tampere, Medical School
Tampere University Hospital, Department of Internal Medicine
Finland

Supervised by
Professor Amos Pasternack
University of Tampere

Reviewed by
Professor Yrjö T. Konttinen
University of Helsinki
Docent Marianne Gripenberg-Gahmberg
University of Helsinki

Distribution

University of Tampere
Sales Office
P.O. Box 617
33101 Tampere
Finland

Tel. +358 3 215 6055
Fax +358 3 215 7685
taju@uta.fi
http://granum.uta.fi

Cover design by
Juha Siro

Printed dissertation
Acta Universitatis Tamperensis 828
ISBN 951-44-5143-0
ISSN 1455-1616

Electronic dissertation
Acta Electronica Universitatis Tamperensis 120
ISBN 951-44-5144-9
ISSN 1456-954X
http://acta.uta.fi

Tampereen yliopistopaino Oy Juvenes Print
Tampere 2001

Contents

Abbreviations

α_1m	alpha-1 microglobulin
AIDS	acquired immune deficiency syndrome
ANA	anti-nuclear antibodies
BE	base excess
BUT	break-up time
β_2m	beta-2 microglobulin
CI	confidence interval
CNS	central nervous system
CRP	c-reactive protein
CTD	connective tissue disease
dRTA	distal renal tubular acidosis
ENA	extractable nuclear antigen
ESR	erythrocyte sedimentation rate
GFR	glomerular filtration rate
GVHD	graft-versus-host disease
HPF	high-power field
IL-6	interleukin-6
KCS	keratoconjunctivitis sicca
LSG	labial salivary gland
MMC	mixed monoclonal cryoglobulins
MCTD	mixed connective tissue disease
NA	not available
NHL	non-Hodgkin lymphoma
PBC	primary biliary cirrhosis
PNS	peripheral nervous system
pRTA	proximal renal tubular acidosis
pSS	primary Sjögren's syndrome
RA	rheumatoid arthritis
RTA	renal tubular acidosis
RF	rheumatoid factor
RNP	ribonucleoprotein
SIR	standardised incidence ratio
SLE	systemic lupus erythematosus
SMR	standardised mortality ratio
SS	Sjögren's syndrome
sSS	secondary Sjögren's syndrome
TIN	tubulointerstitial nephritis
UCTD	undifferentiated connective tissue disease

List of original publications

This thesis is based on the following original publications, referred to in the text by the Roman numerals I–V:

I Pertovaara M, Korpela M, Uusitalo H, Pukander J, Miettinen A, Helin H and Pasternack A (1999): Clinical follow-up study of 87 patients with sicca symptoms (dryness of eyes or mouth, or both). Ann Rheum Dis 58: 423–427.

II Pertovaara M, Pukkala E, Laippala P, Miettinen A and Pasternack A (2001): A longitudinal cohort study of Finnish patients with primary Sjögren's syndrome. Clinical, immunological and epidemiological aspects. Ann Rheum Dis 60: 467–472.

III Pertovaara M, Korpela M, Kouri T and Pasternack A (1999): The occurrence of renal involvement in primary Sjögren's syndrome: a study of 78 patients. Rheumatology 38: 1113–1120.

IV Pertovaara M, Korpela M and Pasternack A: Factors predictive of renal involvement in patients with primary Sjögren's syndrome. Clinical nephrology, in press.

V Hulkkonen J, Pertovaara M, Antonen J, Pasternack A and Hurme M: Elevated interleukin-6 plasma levels are regulated by the promoter region polymorphism of IL-6 gene in primary Sjögren's syndrome (pSS) and correlate with clinical manifestations of the disease. Rheumatology, in press.

1 Introduction

Sjögren's syndrome (SS) is a chronic autoimmune exocrinopathy characterised by dryness of eyes (xerophtalmia) and mouth (xerostomia) as well as recurrent parotid or submandibular gland swellings (Talal 1993). In addition to the glandular symptoms, various extraglandular manifestations in SS have been described (Bloch et al. 1965, Shearn and Tu 1965, Whaley et al. 1973a, Alexander et al. 1982a). The presence of multiple serum autoantibodies is a characteristic feature of the condition (Alspaugh and Tan 1975, Venables et al. 1989). Histologically, lymphocyte and plasma cell infiltration in the affected organs is observed (Talal and Bunim 1964, Bloch et al. 1965, Whaley et al. 1973a).

Sjögren's syndrome is named after a Swedish ophthalmologist, Henrik Sjögren, who in his thesis “Zur Kenntnis von Keratoconjunctivitis sicca” in 1933 reported detailed clinical and histological findings in 19 women with keratoconjunctivitis sicca (KCS) and xerostomia, of whom 13 had chronic arthritis (Sjögren 1933). Bloch and associates (1965) suggested that SS patients should be subdivided into those with sicca symptoms (dryness in the eyes and mouth) alone and those evincing these symptoms in association with other autoimmune connective tissue diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), scleroderma, poly- or dermatomyositis, as well as primary biliary cirrhosis (PBC) (Whaley et al. 1973a, Whaley et al. 1973b). Subsequently, the concepts of primary and secondary Sjögren's syndrome (pSS and sSS) were introduced (Moutsopoulos et al. 1979). However, sicca symptoms are not specific for SS but may occur in a number of other conditions. Research on the outcome of patients with pure sicca symptoms is sparse.

SS has previously been considered an exceedingly rare disorder; Sjögren (1933) estimated the prevalence of sicca syndrome to be 0.05%. Nowadays, an increasing body of evidence would indicate that the prevalence of SS approaches or exceeds that of RA, (Jakobsson et al. 1989, Thomas et al. 1998) which is usually believed to affect from 1 to 3% of the world population. SS is likely to be underdiagnosed, and factors identified as suggestive for a diagnosis might prove helpful in recognising the condition in clinical work.

In most pSS patients lymphoproliferation remains confined to lacrimal and salivary tissue and the disease has a chronic, benign course of stable or progressive xerophthalmia and xerostomia (Talal 1993). However, various systemic manifestations belong to the clinical spectrum of pSS; the data on the frequency of renal involvement in pSS are somewhat contradictory. An increased risk of malignant lymphoproliferative

diseases, especially of non-Hodgkin lymphoma (NHL), has been related to SS (Talal and Bunim 1964, Kassan et al. 1978).

The etiology of SS is unknown, but recent years have brought a clearer understanding of its pathogenesis (Jonsson et al. 1999). Several cytokines, including interleukin-6 (IL-6) have been proposed to be involved (Hagiwara et al. 1998, Halse et al. 1999). Elevated serum IL-6 concentrations have been found in patients with SS (Pettersson et al. 1992, Grisius et al. 1997). The genetic background of this finding and its possible associations with clinical findings of SS have not been studied.

This thesis focuses on the clinical presentation of pSS evaluating the outcome of patients with sicca symptoms and those with pSS, laying special emphasis on the occurrence of renal manifestations in pSS and furthermore, to correlations of IL-6 plasma concentrations and promotor region gene polymorphism with various clinical manifestations of pSS.

2 Review of the literature

2.1 Diagnosis and clinical features of Sjögren's syndrome (SS)

2.1.1 Differential diagnosis of sicca symptoms

Symptoms of dry eyes and mouth may be related to normal ageing (Osterberg et al. 1984, Hochberg et al. 1998, Schein et al. 1999). The use of certain medications (e.g. anticholinergic or antidepressive drugs, diuretics, beta-blockers) may cause xerophthalmia and xerostomia (Schein et al. 1999). Xerophthalmia may be present in local ocular disorders such as conjunctival infections, chronic inflammatory conditions of the eye, and a number of extraocular disorders, i.e diabetes and hypogonadism (Murube-del-Castillo and Cortes-Rodrigo 1989). Several other disease processes, including acquired immune deficiency syndrome (AIDS) (Ulirsch and Jaffe 1987*),* actinomycosis (Rice 1999), alveolitis (Chanana and Sharma 1997), amyloidosis (Gogel et al. 1983), graft-versus-host disease (GVHD), hemochromatosis and lymphoma (Talal 1993), hyperlipoproteinemias IV and V (Goldman and Julian 1977) and sarcoidosis (Drosos et al. 1989) may cause symptoms resembling SS. The symptoms of SS may also overlap with those of other autoimmune diseases, e.g. RA (Bloch et al. 1965), scleroderma (Drosos et al. 1988) and SLE (Andonopoulos et al. 1990). Dry eyes and dry mouth are also considered features of a number of non-specific syndromes such as fibromyalgia (Bonafede et al. 1995) and chronic fatigue syndrome (Calabrese et al. 1994). SS cannot thus be diagnosed solely by clinical symptoms; it is necessary to confirm a presumptive diagnosis of pSS by establishing objective evidence of dry eyes and the presence of focal lymphoid infiltrates in minor salivary glands and autoimmunity in a patient with clinical sicca symptoms.

2.1.2 Classification criteria

Originally, sicca syndrome was defined as a triad of RA or another autoimmune disease, xerostomia and dry eyes (Bloch et al. 1965). Bloch and associates (1965), however, described separately patients with pure sicca symptoms and those with an associated autoimmune disease. Since 1979 SS has been divided into primary and secondary forms (Moutsopoulos et al. 1979). Thereafter, several sets of diagnostic criteria for SS have been proposed, of which five are currently in general use: the Californian (Fox et al.

Table 1. Adapted summary of the Californian, the Japanese, the Copenhagen, the Greek and the European classification criteria for Sjögren's syndrome.

Criteria	Fox et al. 1986	Homma et al. 1986	Manthorpe et al. 1986	Skopouli et al. 1986	Vitali et al. 1993
Ocular component					
1. Subjective	None	Xerophthalmia	None	Xerophthalmia and	Xerophthalmia (Specific questions)
2. Objective	Sch ≤ 9mm/5min and RB positive or Fluorescent +	Sch < 10mm/5min and RB ≥ ++ and/or Fluorescent +	Sch I ≤ 10mm/5min RB: van Bijsterveld score ≥ 4 BUT ≤ 10s	Sch ≤ 5mm/5min or RB positive	Sch I ≤ 5mm/5min or RB: van Bijsterveld score ≥ 4
Oral component					
1. Subjective	Xerostomia and	Xerostomia / RSGS	None	Xerostomia and	Xerostomia / RSGS (Specific questions)
2. Objective	Decreased basal and stimulated SFR	Decreased SFR	Unstimulated SFR ≤ 1.5 ml / 15 min	Parotid flow ≤ 1 cc / 5min or RSGS and	Unstimulated SFR ≤ 1.5 ml / 15 min or
			Scintigraphy: diminished uptake		Scintigraphy + or
		Sialography: diffuse nodular or globular shadows			Sialography +
	LSG ≥ 2 focuses (Greenspan et al. 1974)	LSG > 1 focus	LSG > 1 focus	LSG ≥ 2+	LSG ≥ 1 focus
Laboratory	RF + or ANA + or SSA/SSB-ab +				RF + or ANA + or SSA/SSB-ab +
Exclusions	Pre-existing lymphoma, GVHD, AIDS, sarcoidosis				Lymphoma, GVHD, AIDS, sarcoidosis
Diagnosis of SS:	Definite: 4/4 criteria, no exclusions Possible: 3/4 criteria, no exclusions	Definite: KCS (RB ≥ ++) or findings in lacrimal biopsy or LSG or sialography (≥ 2/3 criteria) Probable: KCS suspected (RB ≥ +) or decreased SFR or RSGS	≥ 2/3 of the oral and ocular tests must be abnormal	Definite: ≥ 2/3 and LSG ≥ 2+ Possible: ≥ 1/3 and LSG ≥ 0	≥ 4/6 criteria, no exclusions

Sch = Schirmer test; RB = Rose-Bengal test; BUT = break-up time; RSGS = recurrent salivary gland swelling; SFR = salivary flow rate; LSG = labial salivary gland biopsy; KCS = keratoconjunctivitis sicca; SS = Sjögren's syndrome; RF = rheumatoid factor; ANA = antinuclear antibodies; GVHD = graft-versus-host disease; AIDS = acquired autoimmune deficiency syndrome

1986), the Japanese (Homma et al. 1986), the Copenhagen (Manthorpe et al. 1986), the Greek (Skopouli et al. 1986), and the European Community Criteria (Vitali et al. 1993) (Table 1). The European criteria are based on a multicenter validation procedure, while the other sets are empirical.

All of these criteria sets entail the establishment of objective evidence of dryness of the eyes and mouth, and all except the Copenhagen criteria also stipulate the presence of subjective sicca symptoms. The Californian criteria are more stringent than the rest, necessitating also evidence of systemic autoimmune abnormalities and a higher focus score in the salivary gland than the European criteria. Thus only about 10 to 15% of the patients fulfilling the European criteria fulfil the Californian criteria (Fox et al. 1998). A more recent abstract by the European Community Study Group has suggested that inclusion of antibody to anti-SSA (Ro) or minor salivary gland biopsy be obligatory for diagnosis (Vitali and Bombardieri 1997).

2.1.3 Glandular features

The most typical clinical features of SS are symptoms of dryness in various glandular tissues: eyes, mouth, nose, posterior pharynx, larynx, respiratory epithelium, skin and vagina (Kassan and Gardy 1978). Diminished tear fluid secretion causes a sandy sensation in the eyes, burning, redness, photosensitivity, eye fatigue, itching and disturbances of vision (Bloch et al. 1965). Salivary insufficiency may cause difficulty in chewing, swallowing and phonation, adherence of food to buccal surfaces, abnormalities of taste or smell, fissures on the tongue, buccal membranes and lips, need for frequent ingestion of liquids, and worsening of dental caries (Talal 1993). Oral Candida albicans colonisation is detected significantly more often in patients with SS than in healthy controls (Rhodus et al. 1997). Diminished salivary flow may also predispose SS patients to ascending sialadenitis (Rice 1999). Parotid gland enlargement, often recurrent and symmetric, occurs in about half of pSS patients (Wang et al. 1993).

2.1.4 Extraglandular features

Several extraglandular manifestations have been described in SS: arthralgias or arthritis (Bloch et al. 1965), myalgias and myositis (Bloch et al. 1965, Whaley et al. 1973a), purpuric skin lesions (Bloch et al. 1965, Whaley et al.1973a), renal symptoms (Shearn and Tu 1965, Talal et al. 1968), Raynaud's phenomenon (Bloch et al. 1965, Skopouli et al. 1990, Youinou et al. 1990), neurological symptoms (Whaley et al. 1973a, Alexander

et al. 1982a, Hietaharju et al. 1990), respiratory symptoms (Bloch et al. 1965, Whaley et al. 1973a, Strimlan et al. 1976, Constantopoulos et al. 1985), symptoms from the gastrointestinal tract (Bloch et al. 1965, Whaley et al. 1973a), lymphadenopathy (Talal et al. 1968, Pavlidis et al. 1982, Kelly et al. 1991), autoimmune thyroiditis (Karsh et al. 1980), and fatigue (Barendregt et al. 1998).

Polyarthralgias are among the most frequent extraglandular manifestations of pSS (Bloch et al. 1965, Kelly et al. 1991, Markusse et al. 1992, Kruize et al. 1996), whereas erosive arthritis seldom occurs (Kruize et al. 1996). In one study polyarthralgia was found in 84% of the 31 pSS patients examined and synovitis in up to 77%, whereas in general the joint symptoms were mild, being intermittent in 68% of those affected (Castro-Poltronieri and Alarcon-Segovia 1983).

Myalgias are also frequent complaints in pSS, appearing in about half of the patients (Markusse et al. 1992, Kruize et al. 1996). The prevalence of inflammatory muscle disease in pSS, however, has varied from only 2.5% to 10% (Bloch et al. 1965, Shearn 1971, Alexander et al. 1981, Alexander et al. 1983). Subclinical histopathological evidence of muscle inflammation, however, is more frequently encountered (Bloch et al. 1965).

The most common cutaneous vasculitic manifestations of SS are palpable purpura and petechial lesions, generally involving the lower extremities (Alexander 1987). Also urticaria-like vasculitic lesions may be encountered, as well as erythematous macular and papular lesions (Alexander 1987). Raynaud's phenomenenon is a common manifestation in pSS (33%) and has been found to precede the development of sicca manifestations in 42% (Skopouli et al. 1990). A high overall frequency of drug allergies has been associated with SS (Bloch et al. 1965, Katz et al. 1991, Tishler et al. 1998a), as well as a specific trimethoprim-associated systemic reaction (Antonen et al. 1999).

Peripheral nervous system manifestations in pSS include mononeuropathies and polyneuropathies, which can present as distal symmetric sensory or sensorimotor polyneuropathies as well as sensory neuronopathies (Whaley et al. 1973a, Alexander et al. 1982a, Hietaharju et al. 1990). Vasculitis may be the most common cause of distal sensory or sensorimotor polyneuropathies in SS (Mellgren et al. 1989), although entrapment neuropathies are also frequently encountered (Hietaharju et al. 1990). Central nervous system affision can appear as focal (psychomotor epilepsy or extrapyradimal signs) or diffuse symptoms (MS-like syndrome, aseptic meningitis) from the brain (Alexander et al. 1981, Hietaharju et al. 1990) and also as symptoms from the spinal cord (Konttinen et al. 1987). Conflicting data have been presented on the existence of autonomic neuropathies in pSS (Andonopoulos and Ballas 1995, Hakala and Niemelä 2000, Niemelä et al. 2000).

Respiratory tract abnormalities in patients with SS include sicca symptoms from the upper airways and tracheobroncial mucous membranes, lymphoid interstitial pneumonitis, interstitial fibrosis, pleurisy, vasculitis, pulmonary pseudolymphomas and lymphomas (Strimlan et al. 1976) as well as small airways disease (Constantopoulos et al. 1985) and hyperinflation (Lahdensuo and Korpela 1995).

Difficulty in swallowing is frequent in pSS patients, due to severe dryness of the esophagus (Hradsky et al. 1967a, Shearn 1977) or impairment in esophageal motility (Ramirez-Mata et al. 1976). Chronic atrophic gastritis is more frequent in pSS than in controls (Maury et al. 1985, Pokorny et al. 1991) and lymphocytic infiltrates have been shown in gastric biopsy samples (Kilpi et al. 1983). Impaired response to secretin and pancreozymin has been encountered, suggesting subclinical pancreatic disease (Hradsky et al. 1967b). Clinical or biochemical evidence of liver disease is found in 5 to 10% of pSS patients (Whaley et al. 1970). In a recent study of 90 consecutive pSS patients, 14% had antibodies to hepatitis C virus (Garcia-Carrasco et al. 1997), and celiac disease has also been observed in up to 15% of pSS patients (Iltanen et al. 1999).

Lymphadenopathy has been noted in 4–20% of pSS patients (Pavlidis et al. 1982, Kelly et al. 1991, Markusse et al. 1992), and the association of autoimmune thyroiditis with pSS is acknowledged (Karsh et al. 1980). Sufferers also report more fatigue than healthy controls (Barendregt et al. 1998), this being in fact among the most frequent systemic features of the syndrome (Kelly et al. 1991, Kruize et al. 1996).

2.1.5 Laboratory findings

A mild normocytic, normochromic anemia occurs in about 25% of SS patients, leukopenia in 30% and thrombocytopenia in 8% (Bloch et al. 1965). An erythrocyte sedimentation rate (ESR) greater than 30 mm/hour is encountered in over 90% (Talal 1993). Some half of these patients have hypergammaglobulinemia (Talal 1993), and cryoglobulinemia, often of the mixed IgM-IgG type, may also be present (Tzioufas et al. 1986). Rheumatoid factor is positive in 52–96% of sera (Bloch et al 1965, Whaley et al. 1973a). Antinuclear antibodies, with a homogeneous or speckled pattern of immuno-fluorescence, are seen in 55–83% of pSS patients (Pavlidis et al. 1982, Pease et al. 1993); antibodies to native DNA are occasionally present in low titers (Whaley et al. 1973a). Antibodies to Ro/SSA occur in 52-66% and to La /SSB in 20–42% (Venables et al. 1989, Kelly et al. 1991) of pSS patients, being associated with extraglandular disease manifestations (Kelly et al. 1991, Pease et al. 1993) and, albeit rarely, with the possibility of a child with congenital heart block (Julkunen et al. 1993). Antibodies against alpha-fodrin (Haneji et al. 1997), Golgi complex (Griffith et al. 1997) and

muscarinic acetylcholine receptors (Bacman et al. 1998) are novel findings in patients with pSS, claimed to have pathogenetic in addition to diagnostic implications in SS. Thyreoglobulin antibodies have been found in 21% of pSS patients (Karsh et al. 1980). Serum beta-2 microglobulin (β_2m) levels may be increased in pSS, particularly in patients with associated renal or lymphoproliferative complications (Michalski et al. 1975).

2.2 Renal manifestations in primary SS (pSS)

2.2.1 General

Interstitial nephritis is the most common renal lesion reported in pSS (Tu et al. 1968). It may result in latent or overt tubular dysfunction (Shearn and Tu 1965, Talal et al. 1968, Shioji et al. 1970). Clinically the condition can be observed as distal renal tubular acidosis (dRTA), nephrocalcinosis, nephrogenic diabetes insipidus or, less commonly, proximal renal tubular acidosis (pRTA) or Fanconi syndrome (Shearn and Tu 1965, Talal et al. 1968, Tu et al 1968, Winer 1993). Interstitial nephritis in SS often occurs concomitantly with dRTA (Winer 1993), but dRTA in SS patients can sometimes also evolve in spite of normal renal histological findings (Tu et al. 1968, Eriksson et al. 1995). Interstitial inflammatory infiltrates with renal insufficiency may also be encountered without evidence of RTA (Tu et al. 1968, Winer et al 1977).

Proton secretory defect is the major pathophysiologic mechanism leading to dRTA. Normally, most bicarbonate is reabsorbed in the proximal tubuli, and hydrogen ions are secreted in the distal nephron. RTA is characterised by hyperchloremic acidosis and a reduced ability to acidify the urine. RTA in pSS may be distal (type I RTA, dRTA) or proximal (type II RTA, pRTA). In dRTA, hydrogen secretion in the distal nephron is deficient, whereas pRTA is characterised by an abnormality in reabsorption of bicarbonate by the proximal nephron (Winer 1993, Eriksson et al. 1995).

The proton pump located in renal tubular cell membranes resembles that in gastric mucosa and salivary gland acinar cells. The H-ATPase in renal tubular cells regulates the active hydrogen ion secretion in the distal nephron. The hydrogen ions secreted by the tubular membrane-associated H-ATPase are bound to ammonium and phosphate in urine. One bicarbonate ion is created for every hydrogen ion secreted. Disturbances in these mechanisms result in dRTA.

dRTA is subdivided into overt (complete) and latent (incomplete) forms. In overt dRTA metabolic acidosis (low blood base excess, BE) is found while the urine is alkaline. In patients with latent dRTA (normal blood BE), inability to acidify the urine

is observed only during induced acidosis, i.e. by an oral ammonium chloride loading test (Wrong and Davies 1959).

RTA in pSS may manifest itself as urolithiasis or nephrocalcinosis, in severe cases also as osteomalacia; without therapy it can also lead to renal insufficiency (Moutsopoulos et al. 1991). Severe potassium depletion, acidosis, a low urinary citrate concentration and high urinary pH are also common clinical findings in pSS patients with dRTA (Kassan and Talal 1987). Severe episodes of hypokalemic periodic paralysis (Raskin et al. 1981, Dowd and Lipsky 1993, Siamopoulos et al. 1994) or even respiratory arrest (Poux et al. 1992) have been described in patients with dRTA due to SS. Both tubulointerstitial nephritis (TIN) and dRTA are risk factors for the development of urolithiasis. Hypocitraturia is common in patients with dRTA (Norman et al. 1978), also in those with dRTA and SS (Eriksson et al. 1996a).

In some patients, SS symptoms may be subclinical. Thus, renal disease preceding the onset of symptoms of SS has been reported (Tu et al. 1968). Of ten patients with urolithiasis and dRTA, anti-SSA antibodies were detected in eight and ANA in four, and a diagnosis of possible SS was established in seven (Eriksson et al. 1996b). In 197 hypocitraturic patients with urolithiasis, ANA was found in 18% of 67 female and in 1.5% of 130 male patients, and anti-SSA antibodies in 16% and 0, respectively (Eriksson et al. 1997).

In addition to RTA, necrosis of renal tubules with resultant hyalinisation of glomeruli and renal insufficiency may also result from interstitial nephritis (Gerhardt et al. 1978). Glomerulonephritis in patients with pSS is rare (Goules et al. 2000), and has been described mainly in case reports (Tu et al. 1968, Moutsopoulos et al. 1978). In patients with sSS, renal disease may develop as a result of the primary disease (e.g. SLE or scleroderma), being in such cases typical of the underlying connective tissue disease (CTD) (Bloch et al. 1965, Sobel et al. 1977).

2.2.2 The occurrence of renal manifestations

2.2.2.1 General

Some authors report renal abnormalities as among the most frequent extraglandular manifestations in SS (Pavlidis et al. 1982). In a retrospective survey (Vitali et al. 1991), however, the frequent occurrence of renal abnormalities in SS was questioned, since only 2% of 104 pSS patients evinced signs of renal involvement as investigated retrospectively by blood urea nitrogen and serum creatinine, creatinine clearance and urinalysis. Somewhat contradictory figures have indeed been reported regarding the

prevalence of renal involvement in patients with pSS, the frequency varying between 2% and 67% in different studies (Bloch et al. 1965, Talal et al. 1968, Whaley et al. 1973a, Pavlidis et al. 1982, Shiozawa et al. 1987, Pokorny et al. 1989, Viergever and Swaak 1991, Vitali et al. 1991, Eriksson et al. 1995). This very considerable variation is related to patient selection and differences in methods of studying renal function. Altogether, the patient groups investigated have been rather small and some studies have been retrospective (Bloch et al. 1965, Pavlidis et al. 1982, Vital et al. 1991). Since the earliest series involved both pSS and sSS patients (Bloch et al. 1965, Talal et al. 1968, Whaley et al. 1973a, Shiozawa et al. 1987), it is possible that the renal lesions reported could be associated with the underlying CTD. Very little prospective quantitative information is available on the presence of proteinuria in SS (Siamopoulos et al. 1992). A summary of previous studies of the occurrence of renal manifestations in SS is presented in Table 2.

2.2.2.2 Renal tubular acidosis (RTA)

Originally, Shearn and Tu (1968) reported three out of ten SS patients tested by ammonium chloride loading test to have impairment in acidification of urine, and Talal and colleagues (1968) noted impairment of urinary acidification in six out of 12 patients with SS, of whom two had sSS. The impairment of urinary acidification was overt in three and subclinical in two SS patients (Talal et al. 1968).

In subsequent studies similarly involving both pSS and sSS patients, the frequency of dRTA was between 35–40%: abnormalities in the ammonium chloride loading test were found in six out of 17 (Shiozawa et al. 1987) and in six out of 15 patients with SS tested (Whaley et al. 1973a).

In the majority of studies specifically investigating patients with pSS, the frequency of dRTA has also been 30–48%: dRTA was found in five out of 15 patients (Siamopoulos et al. 1986), in 12 out of 25 (Pokorny et al. 1989) and in seven out of 21 patients studied by ammonium chloride loading tests (Siamopoulos et al. 1992). However, conflicting results have also been reported. In a prospective study involving 20 randomly selected patients with pSS, abnormal acidification capacity was found in only three (Vitali et al. 1991), and in another prospective study, dRTA was found in only three of the 25 pSS patients investigated (Viergever and Swaak 1991).

The highest prevalence figures for dRTA in pSS are those reported from Sweden: abnormal results in the ammonium chloride loading test were noted in 67% of 27 patients with pSS (Eriksson et al. 1995). However, the patient group was not randomly selected, also involving patients already known to have either dRTA or urolithiasis.

Table 2. Summary of previous studies on the occurrence of renal manifestations in Sjögren's syndrome.

Study	Number and type of patients	Renal manifestation			
		NH_4Cl abnormal	Proteinuria	Renal impairment	Other
Bloch et al. 1965 retrospective	62 (pSS and sSS)				16% hyposthenuria
Shearn and Tu 1968 prospective	10	30%			
Talal et al. 1968 prospective	12 (pSS and sSS)	50%	0		
Tu et al. 1968 prospective, histologic	9		67%	50% (crea-cl)	
Shioji et al. 1970 prospective, histologic	4 with RTA (pSS and sSS)		100%		
Whaley et al. 1973 retrospective	15 (pSS and sSS)	40%			20% concentration defect
Pavlidis et al. 1982 retrospective	47 pSS			38% (high U-pH, low s-HCO_3 or high s-crea)	
Siamopoulos et al. 1986 prospective	15 pSS	30%			
Shiozawa et al. 1987 prospective	17 (pSS and sSS)	35%	18%		
Pokorny et al. 1989 prospective	25 pSS (NH_4Cl) 65 pSS (pu)	48%	 5%		
Vitali et al. 1991 retrospective prospective	 104 pSS 20 pSS	 15%	 	 2% (crea-cl)	
Viergever et al. 1991 prospective	27 pSS	12%	0		25% tubular pu (β_2m)
Siamopoulos et al. 1992 prospective	21 pSS	33%	10%		
Eriksson et al. 1995 prospective	27 pSS	67%		33% (Cr-EDTA-cl)	46% tubular pu (α_1m)

pSS = primary SS; sSS = secondary SS; RTA = renal tubular acidosis; NH_4Cl = ammonium chloride loading test; U-pH = urinary pH, s-HCO_3 = serum bicarbonate; s-crea = serum creatinine; pu = proteinuria; crea-cl = creatinine clearance; β_2m = beta-2 microglobulin; α_1m = alpha-1 microglobulin

2.2.2.3 Renal impairment

Mild azotemia is also possible in patients with SS and has been found to correspond to the severity of the interstitial disease (Tu et al. 1968). In another study, five patients with SS and decreased GFR had TIN, and when the histopathological findings were quantified the GFR values were inversely related to the extent of the tubulointerstitial lesions (Eneström et al. 1995). The impaired acid excretion is, however, out of proportion to the reduction in glomerular filtration rate (GFR) (Shearn and Tu 1968).

In a retrospective study of 47 patients with primary SS signs of renal abnormalities (repeatedly high urine pH, low serum bicarbonate or elevated serum creatinine concentration) were found during the initial evaluation in 26% of the patients and at follow-up in 38% (Pavlidis et al. 1982).

In a retrospective survey (Vitali et al. 1991) only two of 104 pSS patients had decreased 24-hour creatinine clearance values (41 and 60 ml/min). In another study 27 SS patients were investigated by Cr-EDTA clearance test and a lowered GFR was found in up to 33%; in most, however, GFR was only slightly or moderately reduced (Eriksson et al. 1996b). The differences in these two studies may be explained by the fact that 24-hour creatinine clearance and serum concentration of creatinine give less reliable estimates of GFR than Cr-EDTA clearance. On the other hand, the retrospective nature of the first study, and the slightly selected patient material in the other, might have had an impact on the results. Also pSS patients with end-stage renal disease have been described (Gentric et al. 1989, Dabadghao et al. 1995).

2.2.2.4 Proteinuria

In patients with TIN, proteinuria is usually insignificant and the urine sediment findings are often non-specific. Proteinuria occurs in SS (Tu et al. 1968, Shioji et al. 1970, Siamopoulos et al. 1986, Shiozawa et al. 1987, Pokorny et al. 1989, Siamopoulos et al. 1992), but excretion rates greater than 0.5 g/d have been found in less than 3% of patients (Shearn 1971). None of the 12 SS patients with renal abnormalities described by Talal and co-workers (1968) had proteinuria. Viergever and Swaak (1991) found no positive results in their 27 patients tested for albumin with dipstick. On the other hand, Tu and associates (1968) presented histological data on nine SS patients, of whom six had proteinuria, 1.0g/d at the highest. All four patients with RTA described in another histological study (Shioji et al. 1970), had proteinuria, but the amounts were not reported. Tested by a semi-quantitative method proteinuria was found in three out of 17

patients (Shiozawa et al. 1987). Pokorny and colleagues (1989) reported proteinuria in 5% of 65 SS patients and aminoaciduria in 3% of them. Siamopoulos and associates (1992) detected proteinuria greater than 3.5 g/d in two of the 21 patients studied.

Tubular proteinuria is characterised by leakage of small and medium-sized molecules. This can be estimated by measurement of urinary alpha-1 microglobulin (α_1m) or β_2m, or by assessing tubular enzymuria such as N-acetyl-beta-D-glucosaminidase (NAG) or lysozyme. Increased urinary α_1m levels have been related particularly to proximal tubular disorders (Yu et al. 1983) and urinary β_2m excretion is also an indicator of proximal tubular dysfunction (Weise et al. 1981)

Increased urinary β_2m excretion was found in four out of 11 SS patients (36%) in one study (Shiozawa et al. 1987). In a study of 27 pSS patients (Viergever and Swaak 1991), tubular proteinuria measured by urinary β_2m excretion was found in 26%, and altogether various signs of tubular dysfunction in 16 (59%). In another recent study (Eriksson et al. 1995), increased excretion of urinary α_1m was observed in up to 46% of the 24 pSS patients. Most patients with tubular proteinuria or enzymuria had a decreased GFR, probably reflecting a more severe renal injury (Eriksson et al. 1995).

2.2.3 Factors associated with renal findings

In a histological study no differences were found between four SS patients with RTA and ten without this complication with respect to the ages of the patients (Shioji et al. 1970). In two other studies (Shiozawa et al. 1987, Pokorny et al. 1989) the patients with RTA were younger than those with normal acidification.

Conflicting data have been reported on the association of disease duration with renal acidification abnormalities. Shioji and colleagues (1970) found no differences in this respect between SS patients with RTA and those with normal acidification capacity. The duration of the disease in SS patients with RTA has been found to be longer (Shiozawa et al. 1987) and shorter (Pokorny et al. 1989) than in those with normal acidification capacity.

Hypergammaglobulinemia has been associated with dRTA both in SS and in other conditions (Shearn and Tu 1965, Meltzer et al. 1966, McCurdy et al. 1967, Morris and Fudenberg 1967, Talal et al. 1968). In contrast, no significant associations between serum gammaglobulin concentration and dRTA were found in several subsequent studies (Pokorny et al. 1989, Vitali et al. 1991, Viergever and Swaak 1991, Siamopoulos et al. 1992).

Anti-SSA and anti-SSB antibodies have been associated with systemic manifestations of SS (Moutsopoulos and Zerva 1990, Kelly et al. 1991, Pease et al. 1993),

while in other studies no differences have emerged in these antibodies (Pokorny et al. 1989, Siamopoulos et al. 1992) or other seroreactivity (Shioji et al. 1970) between patients with dRTA and normal renal acidification capacity.

A high level of serum β_2m has been associated with abnormal renal findings in SS (Michalski et al. 1975). Viergever and Swaak (1991), on the other hand, found no statistically significant correlation of serum β_2m with abnormal tubular tests.

2.2.4 Renal histopathological findings and pathophysiology

2.2.4.1 Interstitial nephritis

Interstitial nephritis with interstitial lymphocyte infiltration and varying degrees of fibrosis and tubular atrophy is the most common renal histological finding in patients with pSS (Talal et al. 1968, Tu et al. 1968). The renal histological findings thus resemble those seen in the exocrine glands. The development of RTA in patients with SS or related diseases has been held to be caused by hypergammaglobulinemia (McCurdy et al. 1967, Morris and Fudenberg 1967), or to be immunologically mediated (Talal 1971). However, hypergammaglobulinemia as such does not cause impairment of renal acidification. When urinary acidification was tested in RA patients no abnormalities in acidification were found in those with hypergammaglobulinemia (Pasternack et al. 1970). The assumption that lymphocytic and plasma cell infiltrates surrounding renal tubules may cause a renal tubular defect has been supported by a number of studies (Talal et al. 1968, Pasternack and Linder 1970, Shioji et al. 1970, Winer et al. 1977 and Gerhardt et al. 1978).

Pasternack and Linder (1970) found lymphocytic and plasma cell infiltrates surrounding the renal tubules and identified C3 and immunoglobulin by immuno-fluoresence techniques in renal tubular cells in four patients with RTA. Talal and associates (1968) studied pSS patients with renal acidification defects and found interstitial infiltrates in all patients studied and focal glomerulonephritis in one. In one patient with pSS immunofluoresence with IgG antiserum in the renal interstitial infiltrate was shown (Gerhardt et al. 1978), and in another patient focal deposits of IgG and C3 in the renal tubular basement membrane with interstitial inflammation were demonstrated (Winer et al. 1977). Local formation of immune complexes as one of the mechanisms underlying the development of tubulointerstitial disease in SS was proposed. Evidence for an immune pathogenesis of the interstitial nephritis and RTA in SS are the presence of immunocompetent cells in the interstitium and the finding of

immunoglobulin and complement in the tubular cell cytoplasm or in the interstitial cells in patients with SS (Kassan and Talal 1987).

Transient neonatal tubular dysfunction caused by antibodies from mothers with SS has been reported (Jordan et al. 1985), and case reports of patients with SS and dRTA with an absent tubular membrane-associated enzyme H-ATPase have been presented (Cohen et al. 1992, Bastani et al. 1995, DeFranco et al. 1995).

2.2.4.2 Immune complex glomerulonephritis

Both membranous, membranoproliferative (Moutsopoulos et al. 1978) and proliferative (Meltzer et al. 1966, Hardin 1975, Font et al. 1989) glomerulonephritis in SS have been described, as well as a crescentic glomerulonephritis associated with membranous nephropathy (Tatsumi et al. 1998). Recently, mesangioproliferative glomerulonephritis in association with pSS was also reported (Goules et al. 2000).

Moutsopoulos and colleagues (1978) described two patients with membrano-proliferative and one with membranous glomerulonephritis. In two cases, interstitial infiltrative changes were also noted. All three patients had immune complexes and two of them also mixed cryoglobulins in their serum at the time when glomerulonephritis was diagnosed. All evinced extraglandular symptoms with vasculitic skin lesions or purpura, marked hypertension and urinary signs of hematuria and proteinuria. Proliferative glomerulonephritis has been described in cases of mixed cryoglobulinemia in SS (Meltzer et al. 1966, Hardin 1975).

In a recent paper reporting on the occurrence of clinically evident renal involvement in pSS (4%), nine of the 18 patients who underwent renal biopsy had glomerulonephritis (membranoproliferative or mesangioproliferative) in addition to ten patients with interstitial nephritis (Goules et al. 2000).

The pathophysiology of immune complex glomerulonephritis in SS is not known. An increased level of immune complexes is thought to overwhelm the clearance mechanisms of the glomerular mesangium (Moutsopoulos et al. 1978). The patho-genetic role of cryoglobulins is also not clear. Their effect is suspected to be similar to that of the immune complexes, whose deposition at various sites results in the fixation of complement and tissue injury (Kassan and Talal 1987).

2.3 Outcome of patients with sicca symptoms and pSS

2.3.1 General

A diagnostic delay from onset of symptoms to identification of SS has been reported to occur. In a retrospective study of 47 patients with pSS, it was noted that an average of eight years had elapsed before the diagnosis was made (Pavlidis et al. 1982). Only 20% of patients with subsequent SS were diagnosed at their first rheumatological visit, and the diagnostic delay was on the average three years (Markusse et al. 1992).

Studies concerning the premorbid phase of SS are few in number. A prospective study was carried out to determine clinical, serological and biopsy findings in a population of patients with dry eyes (Forstot et al. 1982). Among 45 patients with KCS, xerostomia was observed in 15 but complete SS in only eight. Of the 15 patients with subjective xerostomia, the seven patients who also had objective evidence of SS were younger and they had longer duration of xerostomia than those with only subjective xerostomia. In another study (Kruize et al. 1996) none of the 56 patients with isolated KCS was reported to have developed SS at follow-up 10-12 years after the initial diagnosis. However, the follow-up assessment did not include a second labial salivary gland biopsy. Two out of the 38 patients with KCS attending the follow-up evaluation had developed signs of another organ-specific autoimmune disease, i.e. autoimmune thyroid disease. Ichikawa and colleagues (1988) studied prospectively whether patients with subclinical disease develop sicca symptoms and found the three patients with subclinical disease to develop clinical SS in 6 years.

2.3.2 Ocular manifestations

Although the clinical course of pSS has been regarded as fairly mild, it has been assumed that xerophthalmia would slowly progress with time. An increase in subjective sicca symptoms in the eyes has indeed been reported (Pavlidis et al. 1982, Ichikawa et al. 1988, Markusse et al. 1992). In a retrospective series (Pavlidis et al. 1982) symptoms of dry eyes were reported in 30% of the patients at disease onset and in 91% at the time of diagnosis. In another report sicca symptoms in the eyes were noted at initial evaluation in 78% of 50 patients, and at follow-up (median from symptom onset 9 years) in 90% (Markusse et al. 1992).

In contrast to the above, however, no worsening of the KCS in patients with pSS as objectively tested was observed over a period of several years in a retrospective

study; Schirmer test results remained unchanged and the average ocular findings evaluated by break-up time (BUT) and Rose-Bengal tests improved during the mean follow-up period of 53 months (Kriegsbaum et al. 1988). The improvement in ocular score would either relate to topical treatment or reflect a spontaneous improvement over time. In another study likewise, the proportion of patients with pSS or isolated KCS experiencing inflammatory reaction of the eyes was lower at follow-up than at baseline (Kruize et al. 1996). Furthermore, in a prospective study subjective ocular sicca symptoms increased over time in six pSS and nine sSS patients, but the changes in those symptoms were not associated with objective changes in staining patterns in Rose-Bengal tests or Schirmer test results (Ichikawa et al. 1988).

2.3.3 Oral manifestations

An increase in the frequency of subjective sicca symptoms in the mouth over time has been found in most studies. An increase in the frequency of dry mouth symptoms from 36% of patients at disease onset to 91% at diagnosis, and of salivary gland enlargement in 30% and 62%, respectively, was reported in a retrospective series of 47 pSS patients (Pavlidis et al. 1982). Markusse and associates (1992) noted sicca symptoms in the mouth at initial evaluation in 70% and in 82% at follow-up, and parotidal swelling in 24% and 40%, respectively, in their 50 pSS patients. In a prospective series subjective oral sicca symptoms increased over time but fluctuations in oral symptoms were independent of objective findings such as salivary secretion, sialographical grades or histopathological severities in salivary glands (Ichikawa et al. 1988). In a recent follow-up study concerning salivary and oral symptoms in pSS, the subjective oral symptoms remained prominent while, likewise, relative stability in salivary measurements was noted (Gannot et al. 2000). In another study no significant differences were noted between baseline and follow-up in the frequency of oral symptoms (Kruize et al. 1996).

Only two studies on repeat labial salivary gland biopsies in SS patients have been carried out (Leroy et al. 1992, Jonsson et al. 1993). The lesions were mainly progressive with time. Labial salivary gland biopsies taken on two occasions from 27 patients with pSS, ten with sSS and four control patients at mean intervals of more than four years were evaluated (Leroy et al. 1992). Eight of the nine initially negative analyses in pSS patients were positive at the second sampling. Jonsson and associates (1993) observed for their part morphological progression of sialadenitis in 14 out of 21 patients (67%) with pSS, and in 14 out of 18 patients (78%) with sSS after a mean follow-up time of about three years.

2.3.4 Extraglandular features

In most studies, the frequencies of different extraglandular findings of SS have been reported to increase over time. In a retrospective study the disease in 47 pSS patients was found to extend with time beyond the exocrine glands (Pavlidis et al. 1982). This was most frequently manifested in renal involvement and vasculitis. Arthralgias or arthritis were observed in 30% of the patients at disease onset and in 64% at diagnosis, Raynaud's phenomenon in 21% and 36% and purpura in 9% and 28%, respectively. When findings at the time of diagnosis and after follow-up were compared, the frequency of renal findings, vasculitis, lymphadenopathy, peripheral neuropathy, lung involvement and pseudolymphoma had increased. Lymphoma was not observed in this report (Pavlidis et al. 1982). Markusse and associates (1992) noted arthralgias at initial and follow-up evaluations at a median of nine-year intervals in 94% of their 50 patients, lethargy in 20% and 64%, myalgias in 26% and 54%, and Raynaud's symptom in 36% and 42%.

On average, the most severe extraglandular features in a pSS cohort have been described in a longitudinal study of 100 patients (Kelly et al. 1991). In a median follow-up time of 34 months pericarditis (10%), pleuroparenchymal lung disease (9%), RTA (3%) associated with osteomalacia in one case, and cerebrovascular accidents (2%) were found to develop in some patients. In three patients PBC developed during follow-up. Severe systemic disease was associated with the presence of anti-SSA antibodies (Kelly et al. 1991). Steroid treatment was needed in 18 patients beacause of dyspnea, pericardial disease, vasculitis, polymyalgia rheumatica, PBC or myositis. Pericardiectomy was needed for one patient and one patient required dialysis in addition to prednisolone and azathioprine treatments for interstitial nephritis. One patient was treated with cyclic chlorambucil, vinblastine and prednisolone for massive lymphadenopathy causing stridor. However, improvement of the features of pSS which could not be attributed to therapy also occurred in 12 patients.

This same cohort of 100 patients has been followed further (median 10 years) to establish whether the different autoantibody profiles expressed at presentation could be used to predict outcome (Davidson et al. 1999). Patients who were initially totally autoantibody-negative remained polysymptomatic, but they did not develop systemic complications of SS or other CTD. On the other hand, revised diagnoses including RA, SLE, MCTD and scleroderma were made during the follow-up period in 39% of initially ANA- and RF-positive cases negative for anti-SSA and anti-SSB antibodies.

A more favourable outcome of pSS patients was observed in another long-term follow-up study (Kruize et al. 1996). After 10–12 years of follow-up, only four out of

31 patients with pSS developed new features related to autoimmune disease (rheumatoid arthritis; IgMkappa paraprotenemia and peripheral neuropathy; sclerodactyly and pernicious anemia; Raynaud`s phenomenon), not necessitating treatment with corticosteroids (Kruize et al. 1996). None of 56 patients with isolated KCS was reported to have developed SS during a follow-up of 10–12 years. Only two out of the 38 KCS patients available for follow-up had developed new signs of autoimmune disease, otherwise no changes in the clinical presentation were observed (Kruize et al. 1996).

2.4 Lymphoproliferation and SS

2.4.1 General

Talal and Bunim (1964) first reported an increased incidence of NHL in patients with SS. There were three cases of lymphoma and one case of Waldenström's macroglobulinemia which developed in 58 patients with SS. Since then numerous reports of this association have been published and pSS is often considered to be a link between autoimmune and lymphoproliferative disease (Talal et al. 1967, Kassan et al. 1978, McCurley et al. 1990, Tzioufas and Moutsopoulos 1998). A summary of previous studies on the frequency of lymphoma in patients with SS is given in Table 3.

The autoimmune disease may precede the development of lymphoma by intervals ranging from 0.5 to 29 years (Talal 1993). The proportion of patients with SS who develop malignant lymphoma varies from 4-9% in different studies. This variation may be a reflection of the different criteria used for the diagnosis of SS and differences in length of follow-up.

Kassan and co-workers (1978) studied the incidence of malignancies in a population consisting of 136 female patients with SS. The average period of follow-up was 8.1 years per patient, covering a total of 1,099 person years. In addition, an age-, sex-, race- and time-specific matched control population was used for comparison. Seven cases of NHL were observed during the follow-up period, which was 43.8 times higher than the expected number of 0.16 in the control population. Thus the risk of lymphoma in the SS population was about 6.4 cases per thousand per year.

The results of most subsequent studies are in agreement with these, although in one retrospective follow-up study of 47 patients with pSS no lymphomas were identified in a mean follow-up time of 4.3 years (Pavlidis et al. 1982). The follow-up features of 100 pSS patients after a median follow-up of 34 months have been presented (Kelly et al. 1991); three cases of lymphoma were detected. In a mean follow-up time

Table 3. Summary of previous studies on the frequency of lymphoma in patients with Sjögren's syndrome.

Study	No.of patients	Criteria	Age at dg, yrs	Female:male	Follow-up, yrs	NHL (%)	Incidence/ 1000/year	RR/SIR
Talal and Bunim 1964	58	NA	NA	NA	NA	3 (5)		
Kassan et al. 1978 Epidemiologic	136	Clinical, Laboratory, Histologic	NA	136:0	8, mean 1,099 person yrs	7 (5)	6.4	RR 43.8
Pavlidis et al. 1982 Retrospective follow-up	47	KCS, LSG, Salivary flow	52, mean	42:5	NA	0 (0)		
McCurley et al. 1990 Retrospective, histopathologic	138	KCS, X, CTD (2/3 criteria)	NA	NA	NA	8 (6)		
Kelly et al. 1991 Clinical follow-up	100	Fox et al.1986 (m)	48, median	97:3	3, median	3 (3)		
Markusse et al. 1992 Clinical follow-up	50	Fox et al. 1986 (m)	52, mean	46:4	9, mean	2 (4)		
Pariente et al. 1992 Clinical follow-up	62	Fox et al. 1986	51, mean	60:2	9, mean	4 (6)	6.9	
Pavlidis et al. 1992 Clinical follow-up	120	KCS,X, Salivary flow	NA	NA	2, median	8 (7)		
Pease et al. 1992 Clinical follow-up	48	Fox et al. 1986	48, mean	NA	9, mean	0 (0)		
Zufferey et al. 1995 Retrospective cohort study	56	EC	43, mean	51:5	12, mean	5 (9)		
Tzioufas et al. 1995 Prospective clinical follow-up	103	EC	54, mean	99:4	6, mean	7 (7)		
Kruize et al. 1996 Clinical follow-up	31 pSS	Daniels and Talal 1987	56, mean	29:2	10-12 0,480 person yrs	3 (10)		
	56 KCS		51, mean	37:19	10-12	0 (0)		
Kauppi et al. 1997 Epidemiologic	676	National Hospital discharge register	NA	572:104	8, mean 5,336 person yrs	11 (2)		

Study	No.of patients	Criteria	Age at dg, yrs	Female:male	Follow-up, yrs	NHL (%)	Incidence/ 1000/year	RR/SIR
Valesini et al. 1997 Epidemiologic	331	EC	F: 50, mean M:47, mean	318:31	6, mean 1,756 person yrs	9 (3)	5.1	RR 33.3
Sutcliffe et al. 1998 Clinical follow-up	72	EC	50, mean	68:4	11, mean	5 (7)		
Davidson et al. 1999 Clinical follow-up	100	Fox et al. 1986 (m)	48, median	97:3	10, median	3 (3)		RR 14.4
Voulgarelis et al. 1999 Multicenter, retrospective	765	EC	NA	NA	NA	33 (4)		

yrs = years; NHL= non-Hodgkin lymphoma; RR = relative risk; SIR = standardised incidence ratio; NA = not available; KCS = keratoconjunctivitis sicca; LSG = labial salivary gland biopsy; X = xerostomia; CTD = connective tissue disease; EC = SS criteria presented by Vitali et al. (1993); (m) = modified; F = female; M = male

of 9 years of 50 patients with pSS, one patient developed NHL and another an adenolymphoma (Markusse et al. 1992). Pavlidis and colleagues (1992) found eight out of 120 SS patients to have developed lymphomas in a median follow-up time of 6.5 years, representing a 6.5% incidence. In a retrospective cohort study of 55 patients with SS over a mean follow-up period of 12 years five patients were found to have developed malignant lymphoma (Zufferey et al. 1995). Lymphoid malignancy was the cause of death in three out of 31 pSS patients in another follow-up study covering a total of 480 person years from the onset of symptoms in these patients (Kruize et al. 1996).

2.4.2 Risk factors

The risk of lymphoma in SS was found not to be associated with either age or duration of disease in the epidemiological study by Kassan and associates (1978). A similar risk of lymphoma in pSS and sSS was reported (Kassan et al. 1978). It has been suggested, however, that malignant lymphomas are more common in pSS than in sSS (Tzioufas and Moutsopoulos 1998). In an epidemiologic cohort study of 676 patients with pSS, 709 with sSS and 9469 with RA identified from the Finnish hospitals national discharge register, the SIR of NHL was found to be 8.7 in pSS, 4.5 in sSS and 2.2 in RA (Kauppi et al. 1997).

Usually, patients with SS who develop lymphoid neoplasms have had a disease with several extraglandular manifestations (Talal and Bunim 1964). The risk of evolving lymphoma has been most frequently associated with lymphadenopathy and parotidal swellings (Talal and Bunim 1964, Kassan et al. 1978, Sutcliffe et al. 1998). Hypergammaglobulinemic purpura or leg ulcers have also been associated with lymphoma development in SS (Talal and Bunim 1964, Zufferey et al. 1995, Sutcliffe et al. 1998). In addition, splenomegaly, pulmonary infiltrates and renal insufficiency (Talal and Bunim 1964) as well as previous exposure to cytotoxic agents (Kassan et al. 1978) have been reported as predictive factors for the development of malignant lymphoma in SS.

McCurley and associates (1990) report contrasting results. Eight of their 138 patients with SS followed for 12 years had malignant lymphomas which were chiefly B cell neoplasms, and 1 out of 4 patients with atypical lymphoid hyperplasia progressed to overt lymphoma. Clinical features such as age, duration of disease, extent of lymphadenopathy, splenomegaly or parotid swelling failed to identify the patients at risk for lymphoma.

A fall in levels of serum gammaglobulins, especially of IgM and autoantibodies, has been found to accompany or precede the onset of lymphoid malignancies in patients

with SS (Talal and Bunim 1964, Anderson and Talal 1971). A decrease in serum autoantibodies and the turning of longstanding hypergammaglobulinemia into hypo-gammaglobulinemia may even lead to immunodeficiency and lowered resistance to bacterial infections (Tzioufas and Moutsopoulos 1998).

The risk of evolving lymphoma in SS has been linked to elevated levels of serum β_2m (Talal and Bunim 1964, Michalski et al. 1975). Patients with pSS have a high incidence of mixed monoclonal cryoglobulins (MMC) in their sera (Tzioufas et al. 1986). The presence of cryoglobulins is associated with a higher prevalence of extraglandular disease and anti-SSA antibodies and RF compared to patients without cryoglobulins. During a five-year period, seven out of 18 patients with pSS with MMC developed lymphoma. Six of these seven had MMC prior to the appearance of lymphoma, compared with 12 out of 96 of the patients who did not develop lymphoma (Tzioufas et al. 1996). Monoclonal rheumatoid factor-associated cross-reactive idiotypes have also been associated with lymphoma development in SS (Tzioufas et al. 1996).

Fourteen out of a group of 21 patients with primary SS had monoclonal lambda light chains in their serum (Moutsopoulos et al. 1983). In patients with extraglandular features monoclonal light chains were observed in 100% and in patients with glandular SS in only 22%. In a study of Greek patients, 70% of SS patients with extraglandular involvement had monoclonal immunoglobulins or light chains in their serum (Moutsopoulos et al. 1985). In addition, all patients excreted monoclonal light chains in the urine.

Molecular analysis of the labial salivary glands has suggested that the identification of B-cell monotypia is predictive of the development of lymphoma elsewhere (Jordan and Speight 1996). In three out of ten patients with SS with monotypic plasma cell populations in the labial salivary gland tissue, progression to systemic monoclonal lymphoproliferative disease has been observed (Bodeutsch et al. 1993). Immunoglobulin heavy chain monoclonality in LSG has likewise been noted as a relatively common finding in patients with SS and may prove a useful marker for the development of lymphoma (Jordan and Speight 1996).

2.4.3 Type of lymphoproliferative disorders in pSS

A spectrum of lymphoproliferative disorders has been described in association with SS, but most of the lymphomas complicating the course of SS are derived from B-lymphocytes (Shin et al. 1991). T-cell lymphoma occurring in SS is unusual (Wilke et al. 1984, Isenberg et al. 1987, Schuurman et al. 1987, Rustin et al. 1988, van der Valk et

al. 1989). Case reports of Hodgkin's disease (Martin-Santos et al. 1990, Vivancos et al. 1992), chronic myelomonocytic leukemia (Ponge et al. 1988) and multiple myeloma (Ota et al. 1995) have been reported to occur in the course of pSS.

2.4.4 SS in non-Hodgkin lymphoma (NHL)

The prevalence of SS in patients with NHL has also been investigated. In one study 14 out of 113 patients with untreated NHL fulfilled the Greek criteria for SS (Janin et al. 1992), while in another, 10 out of 103 patients with NHL were found to have possible SS (Morel et al. 1993).

2.5 Mortality in SS

Mortality in SS has been evaluated in only one previous population based cohort study (Martens et al. 1999). In Olmsted, Minnesota, an incidence cohort with SS from 1976-1992 was investigated. Fifty patients with pSS and 24 with sSS were identified. An average of 7.2 years of follow-up was available for patients with pSS and 9.9 years for those with sSS. Compared with the general population, SS patients had increased mortality, and when pSS and sSS were studied separately, the increase was found to involve patients with sSS but not those with pSS (Martens et al. 1999).

2.6 Interleukin-6 (IL-6) and IL-6 promoter region gene polymorphism in pSS

2.6.1 Etiopathogenesis of SS

The etiology of SS is unknown. However, an interaction between constitutional and environmental factors is considered to lead to autoimmunity in SS (Price and Venables 1995). In an appropriate genetic background an unknown triggering factor, probably viral, may lead to the immunologically mediated inflammatory mechanisms which result in the chronic exocrine gland lesions present in SS. T-cell mediated autoimmune responses in the glandular tissue as well as apoptotic events are currently considered to be of major importance in the pathogenesis of the syndrome (Jonsson et al. 1999). The role of anti-SSA and anti-SSB antibodies in the process has not been clarified (Jonsson

et al. 2000). Recent studies suggest that antibodies against muscarinic acetylcholine receptors expressed in salivary and lacrimal glands may have a role in the pathogenesis of impaired glandular function in SS (Bacman et al. 1998). Several cytokines such as IL-1, IL-1 receptor antagonist, IL-6, IL-10 and interferon-γ have been proposed to be involved in the pathogenesis of SS (Llorente et al. 1994, Dubost et al. 1996, Hagiwara et al. 1998, Halse et al. 1999).

2.6.2 IL-6 in SS

IL-6 is a B-cell growth and differentiation factor highly expressed in many autoimmune diseases (Kishimoto 1992). IL-6 also has direct and indirect hematopoietic activity, mainly promoting megakaryocyte maturation, B-cell development and immunoglobulin synthesis (Riechmann et al. 1991, Han et al. 1991). Elevated IL-6 concentrations have been found in the serum (Pettersson et al. 1992, Grisius et al. 1997, Tishler et al. 1998b), saliva (Grisius et al. 1997, Tishler et al. 1999) and tear fluid (Tishler et al. 1998b) of patients with SS. The possible associations of elevated serum IL-6 concentrations with clinical findings of SS have not been studied.

2.6.3 IL-6 (-174) G/C base exchange polymorphism

Recent findings suggest that the transcriptional activity of the interleukin-6 gene and the plasma levels of IL-6 protein are associated with a single G/C base exchange polymorphism located at the 5' flanking region of the IL-6 gene (Fishmann et al. 1998). Allele G homozygotes and allele G/C heterozygotes have been shown to have higher plasma IL-6 levels, higher IL-6 gene transcriptional activity and higher inducible IL-6 responses than allele C homozygous subjects. An imbalance of this base exchange polymorphism is seen in patients with early-onset juvenile arthritis (Fishmann et al. 1998). In SS this base exchange polymorphism has not previously been studied.

3 Aims of the study

The purpose of the present study was:

1. to assess the outcome of patients with sicca symptoms and to establish which factors predict the development of SS in patients with sicca symptoms (I).
2. to assess the outcome of a cohort of Finnish pSS patients, to determine the SIR for lymphomas and the SMR and to establish possible risk factors for lymphoma development in pSS (II).
3. to investigate the occurrence of renal involvement in patients with pSS (III) and to study factors predictive of renal involvement in these patients (IV).
4. to compare IL-6 plasma levels and promoter region polymorphism of the IL-6 gene in pSS patients and in healthy controls and to ascertain possible associations of plasma IL-6 levels and IL-6 gene polymorphism with clinical and immunological characteristics of pSS (V).

4 Subjects and methods

4.1 Subjects

4.1.1 General (I–V)

The records of all 408 patients with sicca symptoms initially examined in the Department of Internal Medicine, Section of Rheumatology, in Tampere University Hospital during the years 1977 to 1992 were reviewed. The pSS diagnoses were assigned retrospectively using the Californian criteria (Fox et al. 1986) for pSS, with the modifications that salivary flow rate measurements were not made and the histological findings were graded on the Chisholm-Mason scale (Chisholm and Mason 1968), grades 3 and 4 being regarded as diagnostic.

Two separate patient cohorts were selected for the studies: one not fulfilling the Californian criteria for pSS for the follow-up study of patients with sicca symptoms (I) (Figure 1) and those fulfilling the criteria for possible or definite pSS for the other studies (II–V) (Figure 2). Patients with sSS were excluded from the studies. For IL-6 plasma level and the promoter region gene polymorphism determinations (V) a control population of normal healthy adults was obtained from the Finnish Red Cross Blood Transfusion Centre, Tampere.

4.1.2 Follow-up study of patients with sicca symptoms (I)

Among the subjects with sicca symptoms from the ten-year period 1977–1986 (259 subjects), those fulfilling two or less of the modified Californian criteria for pSS were included in the follow-up study of patients with sicca symptoms (109 subjects) (I). Of these, 14 had died prior to the control examination. The remaining 95 were invited for re-evaluation. A total of 87 subjects (92 % of those invited) attended the study. They comprised 72 females and 15 males with a median age of 60 years at follow-up (range, 28–80 years) and a median follow-up-time of 11 years (range, 8–17 years).

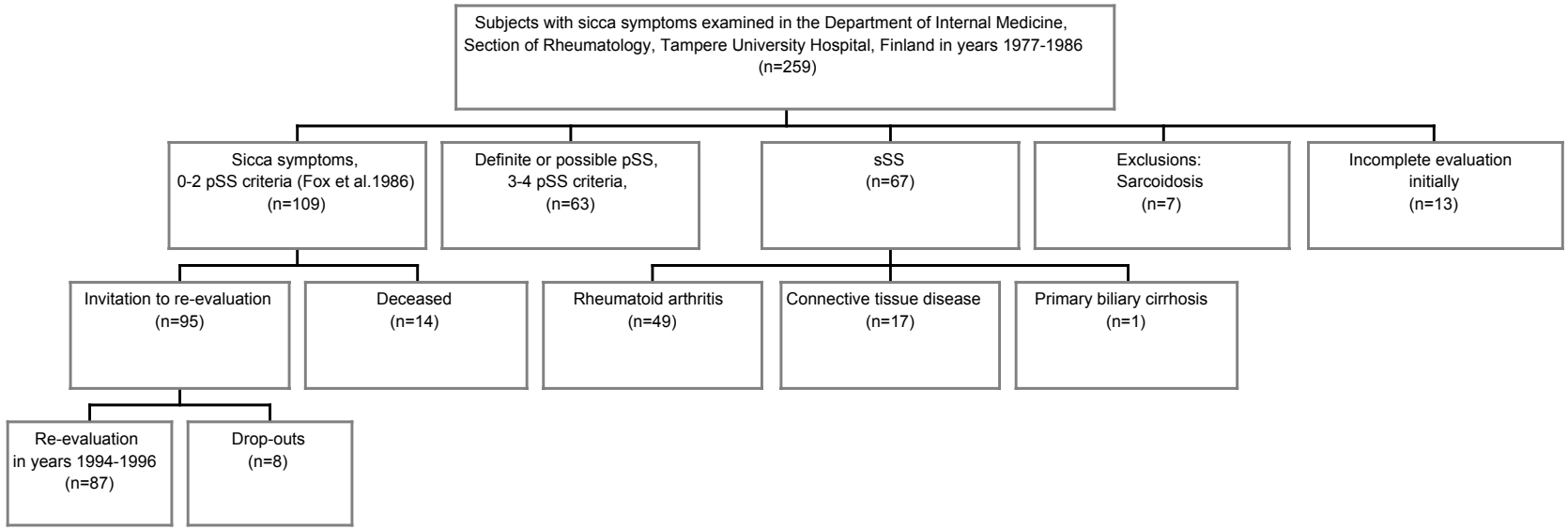

Figure 1. Selection of patients with sicca symptoms (I).

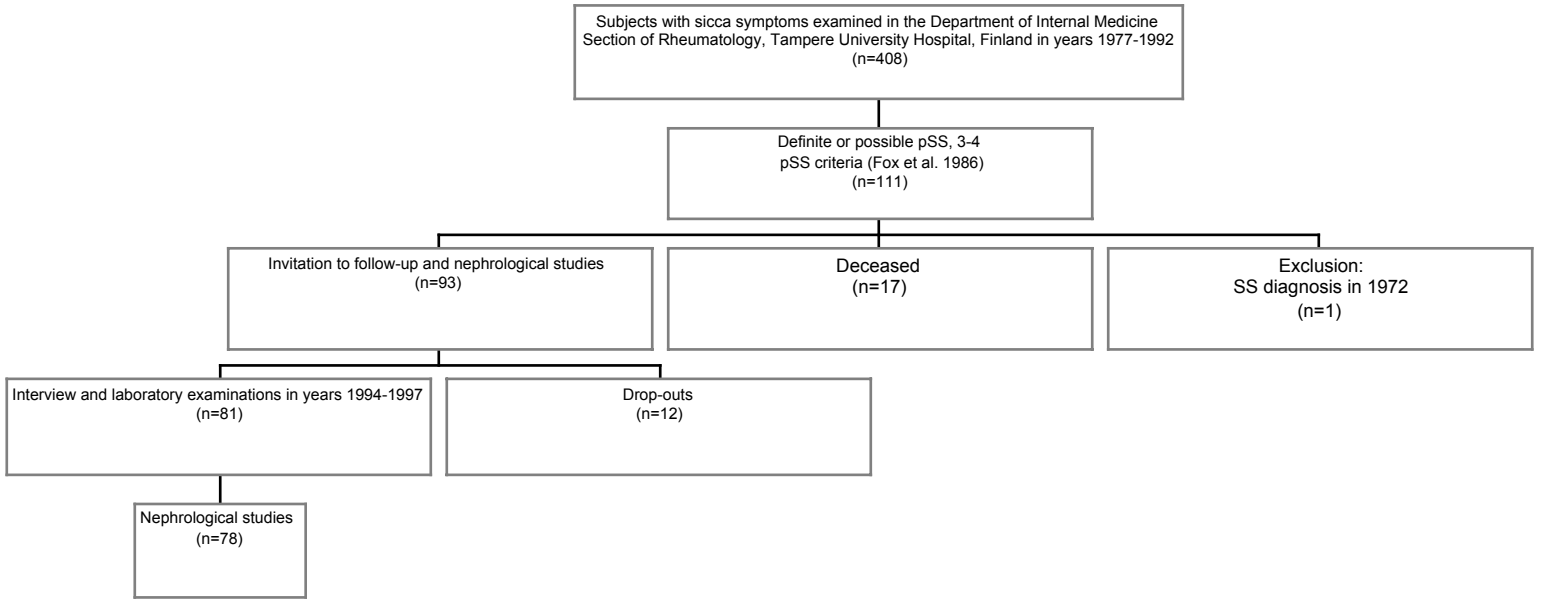

Figure 2. Selection of patients with primary Sjögren's syndrome (II, III, IV).

4.1.3 Cohort study of patients with pSS (II)

One hundred and eleven patients from the years 1977–1992 fulfilled three or more modified Californian criteria for pSS. Of these, one patient with a diagnosis of pSS in 1972 and chronic lymphatic leukemia in 1976, was excluded as the year of diagnosis was before 1977. These 110 patients (108 females, 3 males) comprised the cohort for studies II–V and all of them also fulfilled at least 4 of the European criteria for pSS (Vitali et al. 1993). Seventeen of the 110 patients had died by the time of the control examinations (years 1994–1997). Baseline clinical and laboratory data (from the year of SS diagnosis in each case) on all 110 patients were collected from patient records. The 93 patients still living were invited to attend for interview and clinical and laboratory examinations. Eighty-one of them (87%) complied. In the case of the 12 non-attending patients and those who had died, follow-up data comprised the clinical and laboratory findings from the most recent visit to the rheumatological outpatient clinic. The median age of the 110 patients at the most recent visit was 64 years (range, 29–82 years) and the median disease duration from the time of diagnosis of pSS 9 years (range, 0.1–18 years).

4.1.4 Renal involvement in pSS (III-IV)

Seventy-eight of the 93 pSS patients still living (84%) consented to participate in the nephrological studies (III–IV). The median age of these 78 patients (75 females, 3 males) was 60 years (range, 29–82 years) and the median disease duration from the time of diagnosis of pSS 9 years (range, 3–18 years).

4.1.5 Clinical correlations of IL-6 plasma levels and promoter region gene polymorphism in pSS (V)

The specimens for the IL-6 plasma level and gene polymorphism determinations were obtained after informed consent from 66 pSS patients (64 females, 2 males). The median age of these patients was 62 years (range, 29–82 years) and median disease duration from the time of diagnosis of pSS 9 years (range, 3–18 years). Blood samples from 400 healthy adults (aged 18–60 years) who served as normal controls were obtained from the Finnish Red Cross Blood Transfusion Centre, Tampere.

4.2 Methods

4.2.1 Clinical examination and interview (I–V)

Careful clinical examination and thorough interview of the 87 patients with sicca symptoms and the 81 pSS patients were undertaken (I–V), and furthermore, complementary data were recorded from patient charts. The interview covered family history, previous diseases, previous and concurrent medications, drug allergies, duration of sicca symptoms, first manifestation of the disease, existence of recurrent parotid or submandibular gland swellings, and present sicca symptoms in the eyes and mouth. The existence of xerostomia was defined as a subjective troublesome daily feeling of dry mouth for more than three months; in addition, the presence of a saliva pool at the base of the mouth as well as the dental status were evaluated. In the nephrological studies (III–IV) any history of upper and lower urinary tract infections and renal stones was recorded. The diagnosis of celiac disease was based on small-bowel biopsy histology. Hypertension was defined as previous or current use of antihypertensive drugs. The diagnosis of PBC relied upon liver histology examined with clinical indications.

Special emphasis was focused on extraglandular features of SS (dermatological, endocrine, gastrointestinal, lymphoproliferative, musculoskeletal, neurological, renal, respiratory and vascular symptoms). Purpura was defined as a history of typical episodic palpable purpura lesions in the lower limbs or skin biopsy histology. Lymphadenopathy was defined as lymph node enlargement so persistent as to have indicated a nodal biopsy. Arthritis was defined as articular swelling observed by a clinician. Myositis was diagnosed only if histological findings consistent with inflammation had been found in muscle biopsy. Peripheral and central nervous system (PNS and CNS) symptoms were recorded from the history given by the patients as well as from data from case histories. The diagnosis of pulmonary fibrosis was based on findings in chest radiographs.

4.2.2 Standard laboratory tests and immunological tests (I–V)

The standard laboratory tests included whole blood cell count, erythrocyte sedimentation rate (ESR), serum C-reactive protein (CRP), serum creatinine, serum alanine-aminotransferase and serum alkaline phosphatase (I–IV), serum total protein, serum sodium, potassium and chloride (III–IV).

Rheumatoid factor (RF) was determined at baseline by Waaler-Rose agglutination test (I–II, IV) and at follow-up by laser nephelometry (Behring Nephelometric Analyzer) (I–V). Antinuclear antibodies (ANA) were determined at baseline by indirect immuno-fluorescence on multiblock cryostat sections comprising rat liver and mouse kidney, heart and stomach (I–II, IV) and at follow-up by indirect immunofluorescence using Hep-2 cells (I–V).

Antibodies to extractable nuclear antigens (ENA), including anti-ribonucleo-protein (RNP), anti-Sm, anti-SS-A, anti-SS-B and anti-Scl 70 antibodies, as well as antibodies to native DNA (QUANTA Lite™ ENA 5 ELISA and QUANTA Lite ds DNA, INOVA Diagnostics Inc., San Diego, CA), were measured by enzyme immuno-assay at follow-up (I–V). Anti-salivary gland antibodies were analysed by indirect immunofluorescence (Monkey Salivary Gland Slide, INOVA Diagnostics Inc.) (I–III). Serum concentrations of immunoglobulin IgA, IgG and IgM, as well as serum complement levels (C3 and C4), were measured by laser nephelometry (Behring Nephelometric Analyzer) (I–IV). Serum beta-2 microglobulin (β_2m) was deter-mined by radioimmunoassay (Pharmacia beta-2-micro RIA kit, Pharmacia Diagnostics Uppsala, Sweden) (I–IV).

4.2.3 Ophthalmological examinations (I)

Ophthalmological examination was made at baseline and at follow-up (the latter by one senior ophthalmologist) for the patients with sicca symptoms but not fulfilling diagnostic criteria for pSS. Tear fluid secretion ≤ 9 mm/5 min in Schirmer's test and BUT < 10 sec were defined as abnormal test results. Rose-Bengal test was performed by application of 1% solution of Rose-Bengal dye using a glass applicator and evaluating abnormal staining in the interpalpebral area of the cornea and conjunctiva by slit-lamp. KCS was established if diagnostic criteria (decreased Schirmer test result and abnormal staining in Rose Bengal test) were met in at least one eye.

4.2.4 Labial salivary gland specimens (I)

Labial salivary gland biopsy was performed at baseline in 73 of the 87 patients and the histological findings were graded according to the Chisholm-Mason scale (grades 0–4) (Chisholm and Mason 1968). In the follow-up study of patients with sicca symptoms labial biopsy was repeated for those who initially had grade 0–2 histological findings and who gave their informed consent. A thorough otorhinolaryngological examination

was made and follow-up labial salivary gland biopsy samples via lower lip mucosal incision were taken by a senior otorhinolaryngologist. The histological evaluation was made by an experienced pathologist blind to the clinical data.

4.2.5 Epidemiological methods (II)

The incidence of malignant diseases in the cohort of patients with pSS from the time-point of SS diagnosis up to the end of 1996 through the files of the Finnish Cancer Registry was assessed. The total number of person-years at follow-up was 1,015. The observed incidence of malignant diseases was compared to that expected in the age- and sex-matched population from the same University Hospital district, the reference rates also being calculated from the files of the Finnish Cancer Registry. The dates and causes of death of the pSS patients were assessed from the time-point of SS diagnosis up to the end of 1996 through Statistics Finland

4.2.6 Methods in the nephrological studies (III–IV)

In urinalysis, specimens were screened by dipstick (Combur-10^{M} Test, as read by Miditron Instruments, Roche, Mannheim, Germany), and in cases proving positive for erythrocytes, leukocytes, protein or nitrite, microscopy of the urinary sediment was undertaken according to the Finnish standardisation with Sternheimer stain, where one 400 x high-power field (HPF) equals 0.12 µl original urine volume (Koivula et al. 1990) The healthy upper reference limits were: for women 1 erythrocyte / HPF, 2 leukocytes / HPF, 2 squamous epithelial cells / HPF and for men 1 erythrocyte and 1 leukocyte / HPF and no epithelial cells.

A 24-hour urine collection was conducted and creatinine clearance (enzymatic method, Vitros, Johnson & Johnson, Clinical Diagnostics, Rochester, NY, USA), excretion of urinary total proteins (pyrogallol red method, Olli-C, Kone Instruments, Finland) and immunoglobulin light chains (Behring Nephelometric Analyzer, Marburg, Germany) were determined. Proteinuria was defined as urinary total protein excretion $\geq$ 0.15 g/24 h. The lower reference limit for creatinine clearance for women was 0.94 ml/s/1.73 m^2 and for men 1.32 ml/s/1.73 m^2. Urinary excretion of albumin, IgG and alpha-1 microglobulin (α_1m) was measured from samples obtained in timed (from 10 p.m. to 6 a.m.) overnight urine collection (cU) and analysed by nephelometry (Behring Nephelometric Analyzer, Marburg, Germany). Urinary excretion of albumin $\geq$ 20 µg/min, of α_1m $\geq$ 7.0 µg/min and of IgG $\geq$ 5.0 µg/min were used to discriminate

increased excretion. Lysozyme was also determined from an 8-hour overnight urine collection (cU) and measured using Micrococcus lysodeicticus as standard.

An ammonium chloride loading test was applied as a short-duration test (Backman et al. 1976) provided there was no known contraindication. After waking at 6.30 a.m. the patients voided and at 7.00 a.m. drank 200–400 ml water. At 8.00 a.m. a test meal of ammonium chloride 0.1 g/kg body weight was given in enteric-coated capsels. The test continued until 2.00 p.m. with water supplement of approximately 100 ml/h. Urine samples were taken at the outset and at one-hour intervals up to 6 hours after the test meal. Blood samples were drawn immediately, after 0.5 hour and thereafter at one-hour intervals up to 5.5 hours after the test meal. The voided urine volumes were recorded and samples for urine pH measurement taken in a closed syringe and pH measured with a blood gas analyser (Radiometer ABL 500 Analyzer, Copenhagen, Denmark) within 15 minutes from the sample collection. The samples for determinations of urine titratable acids and ammonium ions were stored in a refrigerator and analysed at the latest on the following day or stored at –20°C and measured within 1 month from collection. The heparinised capillary blood samples were stored in an ice bath and analysed within 1 hour (Radiometer ABL 500 Analyzer, Copenhagen, Denmark). Inability to acidify the urine in maximal acidosis (latent dRTA) was defined according to the reference values related to the degree of acidosis achieved in the acid loading test, as described by Backman and colleagues (1976).

Renal imaging was performed with ultrasonography and with radionuclides using ^{99m}Tc-MAG (mercaptoacetyltriglycine).

4.2.7 IL-6 determination, DNA isolation and genotype analyses (V)

Plasma IL-6 concentrations were determined by commercially available enzyme-linked immunosorbent assay (Pelikine human IL-6 ELISA kit, CLB, Amsterdam) following the manufacturer's instructions. The optical density of individual wells was determined with a "Multiscan Biochromatic 348" (Titertek) spectrophotometer. The detection limit of the assay was 0.6 pg/ml. DNA was isolated from blood samples using the salting out method (Miller et al. 1988). The Nla III polymorphic site at promoter region position –174 of the IL-6 gene was amplified by PCR using oligonucleotides 5'TGACTTCAGCTTTACTCTTGT3' and 5'CTGATTGGAAACCTTATTAAG3' (Fishman et al. 1998). After restriction enzyme digestions the products from PCR analyses were identified by electrophoresis (on 9% PAGE) and ethidium bromide staining. The cycling conditions were analogous to those published (Fishman et al. 1998).

4.2.8 Statistical analysis

Student's t-test and Chi square test or Fisher's exact two-tailed test, as appropriate, were applied for comparisons of continuous and dichotomous variables between different patient groups (I, III–IV).

Student's t-test for paired samples was used to compare differences in means of continuous variables between baseline and follow-up (II). McNemar test was applied to compare dichotomous variables between baseline and follow-up (II). Comparisons between different patient groups were by Mann-Whitney U test and Chi square test or Fisher's exact two-tailed test for continuous and dichotomous variables, respectively (II, V). Findings were considered statistically significant at $p < 0.05$.

Logistic regression analysis in backward stepwise manner was applied to define independent effects of intercorrelated variables on the development of pSS (I) and on the occurrence of RTA and proteinuria in pSS patients (III) and to analyse risk factors for lymphoma development (II).

The relative risk of malignant diseases was calculated using the observed to expected ratio, i.e. standardised incidence ratio (SIR), with 95% confidence intervals and the standardised mortality ratio (SMR) as the ratio of the observed mortality in the cohort to data from Statistics Finland on the expected mortality in the general Finnish population of the same age, sex and time (II).

Statistical analyses were made with the following statistical software: Statview™ (I, III), SPSS 6.1 (I, III–IV) and 7.5 (II) for Windows, Statistica (V).

4.2.9 Ethical considerations

The study protocols were approved by the Ethical Committee of Tampere University Hospital.

5 Results

5.1 The follow-up study of patients with sicca symptoms (I)

5.1.1 General (I)

At follow-up 31 patients (26 females, 5 males) out of 87 (36%) with initial sicca symptoms fulfilled the Californian criteria for SS ("the SS group"), whereas 56 (46 females, 10 males) did not ("the sicca group") (Table 4). Twenty-nine of the patients in the SS group had pSS and two had SS secondary to either RA or mixed connective tissue disease (MCTD) at follow-up. In the sicca group altogether eight patients evinced features of a rheumatic disease at follow-up: two patients fulfilled the criteria for RA, one had undifferentiated connective tissue disease (UCTD), three had features of a seronegative spondylarthropathy and two of a non-specified oligoarthritis.

Table 4. Demographic, clinical and immunological characteristics at baseline among patients with initial sicca symptoms (I).

Characteristic	Sicca group (%)	SS group (%)
Patient number	56	31
Females:males	46:10	26:5
Age at baseline, median (years)	45 (range, 16–71)	53 (range, 28–68)
Sicca symptoms of the eyes	47 (84)	19 (61)
Sicca symptoms of the mouth	35 (63)	24 (77)
Schirmer test result ≤ 9 mm	32 (57)	16 (53) *(n =30)*
Break-up time < 10 sec	10 (30) *(n = 33)*	7 (37) *(n = 19)*
Increased staining in RB test	21 (40) *(n = 52)*	15 (56) *(n = 27)*
Criteria for KCS fulfilled	18 (32)	10 (32)
Grade ≥ 3 in LSG	4 (9) *(n = 45)*	7 (25) *(n = 28)*
RF-positive	9 (16) *(n = 55)*	4 (13)
ANA-positive	2 (3)	8 (26)
Anti-DNA-ab-positive	0 *(n = 48)*	2 (7) *(n = 29)*
S-IgA (g/l)	2.18 ± 0.93	2.46 ± 1.19
S-IgG (g/l)	12.9 ± 4.4	16.6 ± 7.1**
S-IgM (g/l)	1.60 ± 1.43	1.41 ± 0.80
S-gammaglobulin (g/l)	11.8 ± 3.6 *(n = 50)*	15.1 ± 7.3 ** *(n = 28)*
S-β_2m (mg/l)	1.63 ± 0.50	2.15 ± 0.56***

SS = Sjögren's syndrome; RB-test = Rose-Bengal test; KCS = Keratoconjunctivitis sicca; LSG = labial salivary gland biopsy; RF = Rheumatoid factor; ANA = antinuclear antibodies; DNA-ab = antibodies to double-stranded DNA; S-β_2m = serum beta-2 microglobulin Statistical analysis: Student´s t-test and Chi-square test with Yates´ correction. Significance: * $p \leq 0.01$, ** $p \leq 0.005$, *** $p \leq 0.0005$

Patients in the SS group were significantly older than those in the sicca group (63 ± 8 vs. 55 ± 13 years at follow-up, $p \leq 0.005$). The mean duration of dryness of both eyes and mouth was longer in the SS group compared to the sicca group (13 ± 10 and 11 ± 7 years, NS and 13 ± 7 vs. 10 ± 7 years, $p \leq 0.05$, respectively). There were no statistically significant differences in previous or concurrent use of diuretics, beta-blockers, psychiatric medication, tear and salivary substituents, corticosteroids or disease-modifying antirheumatic drugs between the patient groups.

5.1.2 Labial salivary gland biopsies (I)

New labial salivary gland biopsies were taken from 55 patients, and 50 of these were representative (containing more than 4 minor salivary glands / 4 $mm^{2)}$. Biopsy was not undertaken in eleven cases with grade 3 or 4 findings already at baseline. Twenty-one were reluctant to undergo re-biopsy. Of the 42 labial salivary gland re-biopsies of patients with grade 0–2 findings at baseline, progression to grades 3–4 was observed in 21 (50%) at follow-up.

5.1.3 Extraglandular manifestations at follow-up (I)

At follow-up there were no significant differences between the patient groups in the cumulative frequency of extraglandular manifestations such as arthralgias or arthritis, Raynaud's phenomenon, PNS or CNS symptoms or lymphadenopathy. Myositis was not found. Pericarditis had appeared in no patient in the SS group and in two (4%) patients in the sicca group, pleuritis had occurred in six (19%) in the SS group and in two (4%) in the sicca group, but its relationship to SS cannot be ascertained in retrospect. In the SS group two patients (6%) had pulmonary fibrosis and one (3%) had lymphocytic interstitial pneumonitis. One patient (3%) in the SS group had biopsy-proven interstitial nephritis. In the sicca group four patients had developed breast cancer, while in the SS group no malignancies occurred.

5.1.4 Predictive factors for the development of SS (I)

The patients in the SS group had significantly higher mean blood ESR at baseline compared to the sicca group (29 ± 25 vs. 20 ± 19 mm/h, $p \leq 0.05$). The frequency of positive results in the Waaler-Rose test at baseline did not differ significantly between

the groups (Table 4). The SS group had ANA more frequently at baseline than the sicca group (26 % vs. 3 %, $p \leq 0.01$), and the mean concentrations of serum gammaglobulin (15.1 ± 7.3 vs. 11.8 ± 3.6 g/l, $p \leq 0.005$) and serum IgG (16.6 ± 7.1 vs. 12.9 ± 4.4 g/l, $p \leq 0.005$) as well as the level of serum β_2m (2.15 ± 0.56 vs. 1.63 ± 0.50 mg/l, $p \leq 0.0005$) were likewise significantly higher in the SS than in the sicca group at baseline (Table 4).

As several of the clinical and laboratory findings were likely to be intercorrelated, a logistic regression model containing age, ESR, ANA positivity, serum gammaglobulin and IgG concentrations as well as serum β_2m was constructed to assess their independent impact on disease outcome. Age, ANA positivity and serum β_2m were found to be independent predictive factors for the development of SS in subjects with sicca symptoms.

5.2 Cohort study of patients with pSS (II)

5.2.1 Cumulative clinical characteristics and associated diseases (II)

A summary of demographic characteristics and cumulative clinical characteristics and associated diseases in the cohort of 110 pSS patients at follow-up is presented in Table 5. Three of the patients developed NHLs by the end of the follow-up (lymphocytary lymphoma, ileal T-cell lymphoma and diffuse centroblastic centrocytic lymphoma, respectively).

5.2.2 Glandular symptoms and immunoserological findings at baseline and at follow-up (II)

There were no significant differences in the frequency of symptoms of dry eyes (77% vs. 72%) or mouth (94% vs. 88%), or of recurrent salivary gland swellings (39% vs. 46%) between baseline and follow-up examinations in the pSS patients.

Compared with baseline levels there were no statistically significant differences in the follow-up levels of blood hemoglobin, leukocytes, thrombocytes, serum alanine aminotransferase, serum alkaline phosphatase or serum creatinine. Mean blood ESR at follow-up was significantly lower than at baseline (33 ± 22 vs. 45 ± 28 mm/h, $p < 0.0001$). The mean follow-up concentrations of serum protein (77 ± 9 vs. 79 ± 7 g/l, $p = 0.024$), serum IgG (18.8 ± 7.4 vs. 22.5 ± 8.5 g/l, $p < 0.0001$) and serum IgM (1.6 ± 1.1

Table 5. Demographic characteristics and cumulative frequency of clinical characteristics at follow-up of the cohort comprising all 110 patients with primary Sjögren´s syndrome in Tampere University Hospital in the years 1977 to 1992 (II).

Characteristic	Value or frequency (%)
Patient number	110
Females:males	107:3
Age at baseline, median (years)	56 (range, 20–89)
Age at follow-up, median (years)	64 (range, 29–89)
Duration of sicca symptoms of the eyes, median (years)	10 (range, 0–41)
Duration of sicca symptoms of the mouth, median (years)	11 (range, 0–40)
Duration from dg of pSS, median (years)	9 (range, 0–18)
Presence of sicca symptoms of the mouth	97 (88)
Presence of sicca symptoms of the eyes	86 (72)
Arthralgia	82 (75)
Drug allergies	62 (56)
Raynaud´s symptom	55 (50)
Recurrent salivary gland swelling	50 (46)
Myalgia	49 (45)
Persistent dry cough	48 (44)
Sensation of dyspnoe	30 (27)
Peripheral neurological symptoms	23 (21)
Arthritis	24 (22)
Purpura	22 (20)
Lymphadenopathy	17 (16)
Pulmonary fibrosis	17 (16)
Pleurisy	16 (15)
Central nervous system symptoms	12 (11)
Enlarged liver or spleen	10 (9)
Pericarditis	3 (3)
Alveolitis	3 (3)
Renal stones	2 (2)
Overt distal renal tubular acidosis	2 (2)
Renal vasculitis	1 (1)
Child with a congenital complete heart block	1 (1)
Myositis	0

vs. 2.0 ± 1.2 g/l, $p < 0.0001$) were also significantly lower than those at baseline. The change in these parameters could not be attributed to treatment alone. In a subgroup of patients who had never received either corticosteroid therapy or disease-modifying antirheumatic drugs (n = 38) the mean follow-up levels of blood ESR (37 ± 24 vs. 42 ± 24 mm/h, $p = 0.126$, n = 37), serum IgG (20.4 ± 7.6 vs. 23.4 ± 8.9 g/l, $p = 0.003$, n = 35) and serum IgM (1.6 ± 0.6 vs. 2.1 ± 1.0 g/l, $p = 0.014$, n = 35) were lower than those at baseline.

5.2.3 Standardised incidence ratio (SIR) for NHL and other malignancies (II)

By the end of 1996, three NHLs had developed in the 110 pSS patients vs. the 0.23 expected, The SIR of NHL was thus 13 (95% CI 2.7–38). One case of myeloma was noted by the end of 1996, the expected number being 0.12, and SIR was thus 8.7 (95% CI 0.2–48). No cases of Hodgkin's disease were observed. The observed number of all malignancies was not on the whole increased in this patient cohort: eight cases were observed and 7.1 expected, SIR 1.1 (95% CI 0.5–2.2) (Table 6).

Table 6. Standardised incidence ratio of malignant diseases in the cohort comprising all 110 patients with primary Sjögren's syndrome in Tampere University Hospital in the years 1977 to 1992 (II).

Malignancy	Observed	Expected	SIR	95% CI
NHL	3	0.23	13	2.7–38
Myeloma	1	0.12	8.7	0.2–48
Hodgkin's disease	0	0.03	0	0.0–142
Malignant diseases	8	7.1	1.1	0.5–2.2

SIR = Standardised incidence ratio; pSS = primary Sjögren's syndrome; CI = confidence interval; NHL = non-Hodgkin lymphoma

5.2.4 Predictive factors for the development of NHL (II)

The three pSS patients who developed NHL had higher mean baseline concentrations of serum IgA (4.3 ± 0.5 vs. 3.2 ± 1.7 mg/l, p = 0.072), IgM (3.2 ± 0.8 vs. 2.0 ± 1.2 mg/l, p = 0.042) and β_2m (5.40 ± 3.34 vs. 3.16 ± 1.51 mg/l, p = 0.096) and lower mean serum complement C3 concentrations (0.83 ± 0.15 vs. 1.05 ± 0.22 g/l, p = 0.068) than the others.

In a logistic regression analysis including in the model serum baseline immunoglobulin A, G and M concentrations and serum baseline β_2m as well as the baseline age, serum β_2m proved to be an independent predictive factor for lymphoma development in patients with pSS (OR 1.9 with 95% CI 1.1–3.3, p = 0.031).

Clinical glandular findings at baseline: sicca symptoms in the eyes and mouth, history of recurrent salivary gland swellings, the results of the Schirmer or Rose-Bengal tests or the histological grades in LSG biopsies, did not differ significantly in patients with subsequent lymphoma and those without

5.2.5 Standardised mortality ratio (SMR) and causes of death (II)

All deceased patients in this pSS cohort were female. The SMR for female patients here was slightly increased (1.2). The observed number of deaths was 17 and that expected in an age-, sex- and time-matched general Finnish population 14. The expected number of deaths among males was 0.4. In two of the pSS patients malignant lymphoma was the cause of death.

5.3 Renal involvement in pSS (III–IV)

5.3.1 The occurrence of distal RTA (dRTA) and proteinuria (III)

Sixty-three patients with pSS agreed to participate in an ammonium chloride loading test. In nine cases the test failed due to nausea or vomiting. The acidification capacity was abnormal in 18 patients and the prevalence of dRTA was 33 %.

Proteinuria in the range of 0.15 to 0.42 g/24 h was observed in 34 (44%) out of 78 patients. Two of the patients had previously suffered from proteinuria >1g/24 h due to either vasculitis or interstitial nephritis requiring treatment with either corticosteroids or cyclophosphamide. Two of those with mild proteinuria and one with normal urinary protein excretion had diabetes. Immunoglobulin light chain excretion was observed in one patient (lambda light chain). Increased rates of urinary excretion of albumin, α_1m or IgG were observed in nine (12%), nine (12%) and 11 patients (14%), respectively. Increased urine lysozyme excretion was observed in one patient (1.8 mg/l).

5.3.2 Urinalysis, renal function and histological findings (III)

The dipstick for albumin was positive in three out of 78 patients, that for erythrocytes in 13 patients; in the subsequent sediment examination more than 1 erythrocyte/HPF was found in four patients. The dipstick for leukocytes was positive in 21 patients (27%); in subsequent urine microscopy more than 2 leukocytes/HPF were observed in 12 patients. The culture for urine bacteria was positive in ten. Only two patients had symptoms of urinary tract infection; in other cases the bacteruria was asymptomatic.

In nine out of 78 patients (12%) the level of serum creatinine was increased, seven of them aged over 65 years. Creatinine clearance was decreased in 15 (19%) of the patients, all of whom were over 65 years of age.

Renal biopsy had been performed in three cases with clinical indications: overt dRTA and nephrogenic diabetes insipidus with mild renal impairment (serum creatinine

164 μmol/l) and mild proteinuria 0.25 g/24 h (patient no. 1); proteinuria 1.0 g/24 h and mild renal impairment (serum creatinine 173 μmol/l) (patient no. 2); and proteinuria 5.0 g/24 h, microscopic hematuria and renal impairment (serum creatinine 150 μmol/l) (patient no. 3). The corresponding histological findings were mesangial glomerulonephritis, chronic interstitial nephritis and endocapillary, proliferative glomerulonephritis with renal vasculitis, respectively.

5.3.3 Factors associated with the occurrence of dRTA (III)

The duration of xerostomia (15 ± 8 vs. 10 ± 6 years, $p \leq 0.025$) and the duration of pSS from diagnosis (10 ± 4 vs. 8 ± 4 years, $p \leq 0.05$) were longer, and the presence of hypertension (44% vs. 14%, $p \leq 0.05$) more frequent in those with dRTA than in the others. The mean level of serum creatinine was significantly higher in those with dRTA compared to the rest (92 ± 39 vs 78 ± 13 μmol/l, $p \leq 0.025$) and proteinuria was detected more often in the patients with dRTA than in those with normal acidification capacity (67% vs. 27%, $p \leq 0.02$). There were no differences in the occurrence of increased rates of excretion of albumin, α_1m or IgG between the patient groups. The level of serum β_2m was significantly higher (3.27 ± 1.60 vs. 2.60 ± 0.58 mg/l, $p \leq 0.025$) in the patients with latent or overt dRTA compared to those with normal acidification capacity (Table 7).

Table 7. Comparison of clinical and laboratory findings in patients with primary Sjögren's syndrome with overt or latent distal renal tubular acidosis (dRTA) and normal renal acidification capacity (III).

Variable	dRTA (n = 18)	No dRTA (n = 37)	Significance
Age, mean (years)	57 ± 13	57 ± 13	NS
Duration of sicca symptoms of the eyes (years)	12 ± 6	11 ± 7	NS
Duration of sicca symptoms of the mouth (years)	15 ± 8	10 ± 6	$p \leq 0.025$
Duration of the disease (years)	10 ± 4	8 ± 4	$p \leq 0.05$
Frequency of hypertension	8 (44 %)	5 (14 %)	$p \leq 0.05$
S-creatinine (μmol/l)	92 ± 39	78 ± 13	$p \leq 0.025$
Creatinine clearance (ml/s/1.73 m^2)	1.22 ± 0.44	1.37 ± 0.37	NS
Frequency of proteinuria (≥0.15 g/24 h)	12 (67 %)	10 (27 %)	$p \leq 0.02$
cU IgG (≥5.0 μg/min)	2 (11 %)	3 (8 %)	NS
cU albumin (≥20 μg/min)	2 (11 %)	1 (3 %)	NS
cU α_1m (≥7.0 μg/min)	2 (11 %)	2 (5 %)	NS
ANA-positive	17 (94 %)	32 (87 %)	NS
Anti-SSA ab-positive	16 (89 %)	26 (72 %)	NS
Anti-SSB ab-positive	13 (72 %)	18 (50 %)	NS
S-IgG (g/l)	21.7 ± 7.3	19.7 ± 6.7	NS
S-β_2m (mg/l)	3.27 ± 1.60	2.60 ± 0.58	$p \leq 0.025$

dRTA = distal renal tubular acidosis; cU = collected urine = timed overnight collection; α_1m = alpha-1 microglobulin; ANA = anti-nuclear antibodies; S-β_2m = serum beta-2 microglobulin
Statistical analysis: Student´s t-test and Chi-square test with Yates' correction

No statistically significant differences were seen in the occurrence of RF, ANA, anti-SSA or anti-SSB antibodies, nor in the concentrations of serum immunoglobulins IgA, IgG and IgM between the groups (Table 7). Anti-DNA antibodies were detected in only one patient in each group.

To assess independent effects of the possibly intercorrelated variables on the occurrence of RTA, logistic regression analysis by backward stepwise method was applied. Duration of xerostomia, serum β_2m, serum creatinine, presence of proteinuria and of hypertension, and age were included in the model. The parameters independently associated with the existence of RTA in this model were hypertension ($p = 0.0263$), proteinuria ($p = 0.0370$) and duration of xerostomia ($p = 0.0404$).

5.3.4 Predictive factors for dRTA (IV)

The mean baseline concentration of serum IgG tended to be higher in those with dRTA (25.6 ± 8.2 vs. 21.9 ± 7.3 g/l, $p = 0.102$) than in those with normal acidification capacity, while the mean baseline concentrations of serum IgA or IgM did not differ between the patient groups (Table 8). The mean levels of total serum gammaglobulin (24.1 ± 7.4 vs. 18.8 ± 6.0 g/l, $p = 0.011$) and total serum protein (84 ± 7 vs. 79 ± 7 g/l, $p = 0.024$) were significantly higher at baseline in those with subsequent dRTA than in those with normal renal acidification capacity. The baseline levels of serum β_2m were higher in the patients with subsequent dRTA (3.07 ± 1.11 vs. 2.56 ± 0.78 mg/l, $p = 0.072$). There were no differences in the occurrence of seropositivity in the Waaler-Rose test or ANA at baseline between those with dRTA and those with normal renal acidification capacity (Table 8).

Clinical baseline findings: sicca symptoms in the eyes and mouth, history of recurrent salivary gland swellings, the results of the Schirmer or Rose-Bengal tests or the histological grades in LSG biopsies did not differ significantly in patients with subsequent overt or latent dRTA and those with normal acidification capacity (Table 8).

5.3.5 Factors associated with the occurrence of proteinuria (III)

The duration of xerostomia was significantly longer in pSS patients with mild proteinuria than in those with normal urinary protein excretion, but there were no statistically significant differences in the duration of sicca symptoms in the eyes nor in the duration of pSS from the year of diagnosis (Table 9). The frequency of hypertension did not differ between the groups, but both systolic and diastolic blood pressures

Table 8. Baseline characteristics in patients with primary Sjögren's syndrome with latent or overt distal renal tubular acidosis and normal renal acidification capacity (IV).

Variable		dRTA (n=18)	No dRTA (n=37)	Significance
Age at baseline (mean ± SD, years)		47 ± 12	49 ± 12	0.487
Sicca symptoms of the eyes		13 (72 %)	26 (70 %)	
Sicca symptoms of the mouth		17 (94 %)	31 (84 %)	
Schirmer test result ≤ 9 mm/5 min		15 (83 %)	33 (89 %)	
Increased staining in Rose-Bengal test		16 (94 %) *(n=17)*	28 (85 %) *(n=33)*	
LSG grade:	0	0	0	
	1	1 (6 %)	4 (11 %)	
	2	6 (33 %)	8 (22 %)	
	3	3 (17 %)	6 (16 %)	
	4	8 (44 %)	19 (51 %)	
	Total	18 (100 %)	37 (100 %)	
History of salivary gland swelling		7 (39 %)	15 (41 %)	
S-creatinine (µmol/l)		84 ± 13 *(n=34)*	80 ± 12	0.358
RF-positive		11(69 %) (*n=16)*	15 (45 %) *(n=33)*	0.125
ANA-positive		8 (44 %)	25 (68 %)	0.100
S-protein (g/l)		84 ± 7 *(n=15)*	79 ± 7 *(n=33)*	0.024*
S-gammaglobulin (g/l)		24.1 ± 7.4 *(n=16)*	18.8 ± 6.0 *(n=32)*	0.011*
S-IgA (g/l)		3.07 ± 1.27	3.09 ± 1.62	0.956
S-IgG (g/l)		25.6 ± 8.2	21.9 ± 7.3	0.102
S-IgM (g/l)		2.29 ± 1.29	1.91 ± 1.03	0.257
S-β_2m (mg/l)		3.07 ± 1.11 *(n=15)*	2.56 ± 0.78 *(n=34)*	0.072

dRTA = distal renal tubular acidosis; LSG = labial salivary gland histology, Chisholm-Mason (1968) scale; RF = rheumatoid factor; ANA = anti-nuclear antibodies; S-β_2m = serum beta-2 microglobulin
Statistical analysis: Student´s t-test and Chi square test or Fisher´s exact test

Table 9. Comparison of clinical and laboratory findings in patients with primary Sjögren's syndrome with proteinuria and normal urinary protein excretion (III).

Variable	Urinary total protein excretion (g/24 h) ≥0.15 (n=34)	<0.15 (n =44)	Significance
Age, mean (years)	59 ± 13	58 ± 13	NS
Duration of sicca symptoms of the eyes (years)	11 ± 7	11 ± 7	NS
Duration of sicca symptoms of the mouth (years)	14 ± 9	11 ± 6	p ≤ 0.05
Duration of the disease (years)	10 ± 4	9 ± 5	NS
Frequency of hypertension	9 (27 %)	9 (21 %)	NS
Systolic blood pressure (mm Hg)	143 ± 20	134 ± 16	p ≤ 0.025
Diastolic blood pressure (mm Hg)	86 ± 11	82 ± 9	p ≤ 0.025
S-creatinine (µmol/l)	88 ± 31	81 ± 19	NS
Creatinine clearance (ml/s/1.73 m^2)	1.21 ± 0.46	1.33 ± 0.41	NS
dRTA	11 (52 %) (*n=21)*	6 (18 %) (*n=33)*	p ≤ 0.02
Microscopic hematuria	2 (6 %)	2 (5 %)	NS
cU IgG (≥5.0 µg/min)	8 (24 %)	3 (7 %)	NS
cU albumin (≥20 µg/min)	9 (26 %)	0	p ≤ 0.01
cU α_1m (≥7.0 µg/min)	7 (21 %)	2 (5 %)	NS
ANA-positive	28 (82 %)	38 (86 %)	NS
Anti-SSA ab-positive	25 (76 %)	32 (73 %)	NS
Anti-SSB ab-positive	21 (64 %)	20 (46 %)	NS
S-IgG (g/l)	19.7 ± 7.7	18.3 ± 6.6	NS
S-β_2m (mg/l)	3.33 ± 1.71	2.73 ± 0.77	p ≤ 0.025

dRTA = distal renal tubular acidosis; cU = collected urine = timed overnight collection; α_1m = alpha-1 micro-globulin; ANA = anti-nuclear antibodies; S-β_2m = serum beta-2 microglobulin
Statistical analysis: Student's t-test and Chi-square test with Yates' correction

measured at the study examination were significantly higher in those with proteinuria (Table 9). dRTA occurred more frequently in those with mild proteinuria than in those with normal urinary protein excretion (52% vs. 18%, $p \leq 0.02$). The levels of serum β_2m were significantly higher in those with proteinuria compared with those with normal urinary protein excretion (3.33 ± 1.71 vs. 2.73 ± 0.77 mg/l, $p \leq 0.025$).

No differences were seen between the proteinuric and non-proteinuric patients in the occurrence of RF, ANA, anti-SSA or anti-SSB antibodies nor in the concentrations of serum immunoglobulins (Table 9). Anti-DNA antibodies were detected in one proteinuric patient and in five patients with no proteinuria; none of them fulfilled more than three of the 11 criteria for SLE (Tan et al. 1982).

A logistic regression analysis was made with duration of xerostomia, serum creatinine level, serum β_2m and systolic and diastolic blood pressures, as well as age in the model. Duration of xerostomia and diastolic blood pressure were found to be independently associated with the presence of proteinuria ($p = 0.0230$ and $p = 0.0126$, respectively) in pSS patients in this model.

5.3.6 Predictive factors for proteinuria (IV)

The mean baseline levels of serum β_2m tended to be higher in patients with mild proteinuria than in those with normal urinary protein excretion (3.10 ± 1.44 vs. 2.52 ± 0.75 mg/l, $p = 0.052$). The baseline levels of serum β_2m were significantly higher in patients with increased excretion of urinary α_1m (4.6 ± 1.8 vs. 2.6 ± 0.8 mg/l, $p = 0.029$) as compared to those with normal urinary α_1m excretion.

There were no significant differences in the mean baseline levels of serum creatinine, serum total protein or concentrations of immunoglobulins A, G or M between those with proteinuria and those without. There were likewise no significant differences between the groups in the number of patients with positive Waaler-Rose test or ANA at baseline (Table 10).

Clinical baseline findings: sicca symptoms in the eyes and mouth, history of recurrent salivary gland swellings, the results of the Schirmer or Rose-Bengal tests or the histological grades in LSG biopsies did not differ significantly in patients who later developed proteinuria as compared to those who did not.

Table 10. Baseline characteristics in patients with primary Sjögren´s syndrome with proteinuria and normal urinary protein excretion (IV).

Variable	Urinary total protein excretion (g/24 h) ≥0.15 (n=34)	< 0.15 (n=44)	Significance
Age at baseline (mean ± SD, years)	49 ± 12	50 ± 12	0.795
S-creatinine (µmol/l)	82 ±14	81 ± 12 *(n=41)*	0.777
RF-positive	20 (67 %) *(n=30)*	16 (43 %) *(n=37)*	0.056
ANA-positive	21 (62 %)	30 (68 %)	0.555
S-protein (g/l)	79 ± 8 *(n=30)*	79 ± 7 *(n=39)*	0.933
S-gammaglobulin (g/l)	21.3 ± 7.7 *(n=28)*	18.4 ± 6.6 (*n=38)*	0.110
S-IgA (g/l)	3.06 ± 1.73	3.08 ± 1.60	0.960
S-IgG (g/l)	23.0 ± 6.9	20.9 ± 8.1	0.237
S-IgM (g/l)	1.96 ± 1.08	2.13 ± 1.23	0.530
S-β_2m (mg/l)	3.10 ± 1.44 *(n=30)*	2.52 ± 0.75 *(n=38)*	0.052

RF = rheumatoid factor; ANA = anti-nuclear antibodies; S-β_2m = serum beta-2 microglobulin
Statistical analysis: Student´s t-test and Chi square test or Fisher´s exact test

5.4 IL-6 and promoter region gene polymorphism in pSS (V)

5.4.1 Clinical associations of IL-6 plasma levels (V)

IL-6 plasma levels were higher in pSS patients compared to those in healthy controls (4.05 ± 2.62 vs. 1.80 ± 2.53 pg/ml, $p < 0.0001$) (Table 11). Furthermore, the plasma IL-6 levels were higher (4.81 ± 2.43 pg/ml) in patients with definite pSS (four Californian criteria) than in those with possible pSS (three criteria) (3.39 ± 2.63 pg/ml, $p < 0.05$). Plasma IL-6 levels were also significantly higher in patients with histological grade ≥ 3 in LSG biopsy (4.47 ± 2.50 pg/ml, n = 49) compared to those with grade < 3 (2.78 ± 2.62 pg/ml n = 17, $p < 0.005$).

Plasma IL-6 levels were significantly higher in pSS patients with associated celiac disease, in patients with PNS symptoms and in patients with lung fibrosis or alveolitis as compared to those without these findings (Table 11). The plasma IL-6 levels were lower in patients with purpura than in those without. No significant differences in IL-6 concentrations were found when the patients were divided into groups by existence of joint pain or swelling, Raynaud's phenomenon, CNS symptoms, hypothyroidism, diabetes, overt or latent dRTA, lymphadenopathy.

Anti-SSA antibody-positive patients had lower IL-6 concentrations than those proving anti-SSA antibody-negative (Table 11). No correlation was found between IL-6 plasma levels and age, disease duration, RF positivity or anti-SSA or anti-SSB antibody titers.

Table 11. IL-6 plasma levels and frequencies of the IL-6 gene-174 polymorphism alleles in healthy controls and in patients with primary Sjögren's syndrome with various clinical manifestations of the disease (V).

Groups	Plasma IL-6 pg/ml (mean ± SD)	n	Significance	G carrier (%)	n	Significance
Normal controls	1.80 ± 2.53	400		282 (71)	400	
pSS patients	4.05 ± 2.62	66	< 0.0001	41 (67)	61	NS
pSS and celiac disease	6.74 ± 2.82	8		6 (86)	7	
pSS, no celiac disease	3.66 ± 2.38	58	< 0.005	35 (65)	54	NS
pSS and PNS symptoms	5.61 ± 1.96	13		10 (91)	11	
pSS, no PNS symptoms	3.65 ± 2.63	53	< 0.005	31 (62)	20	NS
pSS, lung fibrosis or alveolitis	5.62 ± 2.50	8		8 (86)	7	
pSS, no lung fibrosis or alveolitis	3.82 ± 2.58	58	< 0.05	35 (65)	54	NS
pSS and purpura	2.57 ± 1.39	12		7 (64)	11	
pSS, no purpura	4.36 ± 2.72	54	< 0.05	34 (68)	50	NS
pSS and anti-SSA ab	3.55 ± 2.26	45		25 (63)	40	
pSS, no anti-SSA ab	5.41 ± 3.03	19	< 0.05	15 (79)	19	NS
No. of pSS criteria (Fox et al. 1986)						
4 criteria	4.81 ± 2.43	30		22 (67)	33	
3 criteria	3.39 ± 2.63	36	< 0.05	19 (68)	28	NS
Grade in LSG (Chisholm-Mason scale)						
4	4.52 ± 2.55	34				
3	4.37 ± 2.47	15				
2	3.01 ± 2.82	14				
1	1.60 ± 1.05	2				
0	1.87	1	< 0.005[1]			

pSS = primary Sjögren's syndrome; PNS = peripheral nervous system; LSG = labial salivary gland

Statistical analysis: Comparisons between plasma IL-6 concentrations in different groups were performed by Mann-Whitney U test and between allele frequencies by Chi square test and Fisher´s exact test. [1] Comparison between non-diagnostic (0–2) and diagnostic (3–4) grades

5.4.2 Clinical associations of IL-6 (-174) G/C base exchange polymorphism (V)

The allele frequencies were similar in pSS patients and in controls (Table 11). The IL-6 plasma levels were higher in pSS patients with allele G/G (5.35 ± 3.01 pg/ml) than in those with G/C (3.96 ± 2.71 pg/ml) or C/C (3.52 ± 2.40 pg/ml; G/G vs. C/C, $p<0.05$). IL-6 plasma levels did not differ in healthy controls of different IL-6 genotypes. Allele G of the IL-6 gene was more frequently observed in pSS patients with associated celiac disease, PNS symptoms, lung fibrosis or alveolitis and anti-SSA antibody negativity, although the differences did not attain statistical significance.

6 Discussion

6.1 Patient selection (I–V)

As several different classification criteria for SS are in use (Fox et al. 1986, Homma et al. 1986, Manthorpe et al. 1986, Skopouli et al. 1986, Vitali et al. 1993), controversy prevails as to the most preferable set for the diagnosis of SS. Patients for the present studies were originally selected by modified Californian criteria from the patient records of a University Hospital. Thereby, a certain selection had already taken place prior to invitations to participate in the present studies; these are not population-based studies, but the patients were selected from a hospital that serves as a secondary and tertiary referral center.

The Californian criteria (Fox et al. 1986) for pSS are in use in the United States. They comprise the strictest of the sets, necessitating evidence of systemic autoimmune abnormalities and a higher focus score in the LSG biopsy than the European criteria (Vitali et al. 1993). However, the preliminary European Community study group criteria have also recently been modified: inclusion of anti-SSA antibody or minor salivary gland biopsy has been proposed as obligatory for diagnosis (Vitali and Bombardieri 1997).

The European criteria are the only criteria for SS based on a multicenter validation procedure. However, the validation process in question has been criticised (Daniels and Whitcher 1995). The criteria offer a range of diagnostic alternatives (replacement of the LSG histology by salivary scintigraphy, sialography or salivary flow measurement), this leading to a larger and possibly more heterogeneous group of patients with a diagnosis of SS (Daniels and Whitcher 1995). The European criteria include specific questions on the presence of sicca symptoms in the eyes and mouth and could thus not have been used in the process of evaluating patient records retrospectively.

In the follow-up study of subjects with sicca symptoms (I) the use of the European criteria instead of the Californian in the diagnosis of SS would most certainly have increased the number of subjects fulfilling the criteria at follow-up. In addition, however, it would have excluded some subjects at baseline, thus not after all necessarily influencing the proportion of patients fulfilling the diagnostic criteria at follow-up. The most important issue in a follow-up study in this respect seems to be the use of exactly the same criteria at both evaluations.

Nonetheless, as the European criteria are currently almost without exception used in Europe, it was subsequently ascertained that all the pSS patients attending studies II-V also in fact met at least four of the European criteria. Since, however, patients were

originally selected by modified Californian criteria, some of those excluded from the studies would very probably have fulfilled the European criteria. Thus the findings in the nephrological studies can be deemed specific for SS, as patients with only subjective disease were not included; the most disease-specific criteria were applied. On the other hand, the particular aim of the study was to determine the occurrence of renal involvement in patients with pSS, and patients with sSS were carefully excluded. However, the prevalence of renal involvement as well as of NHLs is probably higher in patients from the clinical setting of a University Hospital than would be the case in a population-based material.

In the present studies, two modifications were made to the Californian criteria for practical and ethical reasons. For the first, salivary flow rate measurements were not available, and the existence of xerostomia was thus defined as a subjective troublesome daily feeling of dry mouth for more than three months. Grade 3 histology on the Chisholm-Mason scale was accepted as a diagnostic histology, as new labial salivary gland biopsies for subjects who already had a focus of lymphocytes found in their biopsy were considered unjustified (I). The effect of these modifications on the results is probably not substantial, since LSG histology is generally considered more reliable in evaluating the salivary component of SS than salivary flow measurements and, on the other hand, the proportion of patients with grade 3 histology was small.

6.2 Methods (I–V)

The proportion of patients attending the interview and clinical and laboratory examinations (I–IV) was high; 92% in the follow-up study of subjects with sicca symptoms, 87% in the cohort study of patients with pSS and 84% in the nephrological studies. Since all the remaining pSS patients had also visited the rheumatological outpatient clinic of Tampere University Hospital for regular check-up, the clinical and laboratory data on these patients could also be fairly reliably obtained (II).

In the present follow-up studies (I–II) the baseline data were collected retrospectively from the patient records. However, in our clinic the diagnosis of SS has been made since the 1970s in a highly standardised manner including an ophthalmological examination, labial salivary gland biopsy and a systematic laboratory evaluation, which makes the evaluation of the baseline data in these studies eminently reliable. To avoid interobserver variation the clinical examination and a structured interview of the patients (I–V), the ophthalmological examinations (I) and the repeat labial biopsies (I) at follow-up were undertaken by one rheumatologist, by one ophthalmologist and by

one otorhinolaryngologist. The histological evaluation of all the LSG specimens at follow-up was made by an experienced pathologist blind to the clinical data (I).

The methods for rheumatoid factor and ANA determinations (I-IV) were different at baseline (Waaler-Rose agglutination test and indirect immunofluorescence on multiblock cryostat sections, comprising rat liver and mouse kidney, heart and stomach, respectively) and at follow-up (laser nephelometry and indirect immunofluorescence using Hep-2 cells, respectively), a limitation which should be borne in mind when the proportions of patients seropositive at different time-points are reported.

Anti-ENA antibody determinations were comprehensively available only at follow-up. In our patients the overall occurrence of both anti-SSA and anti-SSB antibodies was higher than in other series (Alexander et al. 1982b, Venables et al. 1989, Moutsopoulos and Zerva 1990). This is probably due to the sensitive ELISA method employed; on the other hand, as anti-SSB antibodies have been found specific to and predictive of SS (Venables et al. 1989, Isenberg et al. 1982), their frequent occurrence also reflects the fact that patients with sSS have been excluded from these studies (II-V).

For evaluation of tubular proteinuria the urinary α_1m excretion rate was determined. Urinary α_1m is superior to urinary β_2m in screening for tubular abnormalities, in particular by merit of its stability at low pH (Yu et al. 1983). Urinary β_2m loses its antigenicity at pH < 6.0, which can lead to underestimation of the intensity of low-molecular-weight proteinuria.

In an overt syndrome of RTA the diagnosis can be readily made from the concomitant metabolic acidosis and alkaline urine, while in latent forms of dRTA there is no systemic acidosis and the only means of disclosing the acidification defect is by an acid loading test. Wrong and Davies (1959) showed that a short ammonium chloride loading in the investigation of renal acidification capacity gives similar information to that obtained after loading over several days. Their material and later studies of normal adults (Morris and Fudenberg 1967, Adler et al. 1968) lack data on the degree of acidity in urine in relation to the degree of acidosis obtained. Backman and colleagues (1976) have emphasised that it should be ensured that acidosis is achieved, as they found that both ammonium chloride capsules and tablets failed to be absorbed in some subjects. As the acidosis achieved in their study was high they could show that the renal excretion of H^+ as titratable acid and NH_4^+ increased with the degree of acidosis, contrary to another report (Tannen 1971). The critical range for normal acidification of urine appeared to lie between a base excess of –3 to –6 mmol/l, below which level all normal subjects had acidified their urine to a pH lower than 5.0.

An ammonium chloride loading test was applied as a short-duration test (Backman et al. 1976) (III) provided there was no known contraindication. Inability to

acidify the urine in maximal acidosis (latent dRTA) was defined (III) according to the reference values (Backman et al. 1976) related to the degree of acidosis achieved in the acid loading test.

6.3 Follow-up study of patients with sicca symptoms (I)

6.3.1 General (I)

Thirty-six per cent of the subjects with complaints of dryness of either eyes or mouth, developed SS after a median follow-up time of 11 years. Furthermore, eight out of the 56 (14%) other patients with initial sicca symptoms developed features of another rheumatic disease. Very few follow-up studies have hitherto been made on patients with sicca symptoms.

The purpose of the follow-up study of patients with sicca symptoms was to obtain further information on the natural history of pSS. The proportion of patients with sicca symptoms who finally developed clinically evident SS was fairly small in spite of the long follow-up time. This would imply that the development of SS is probably a particularly slow process. On the other hand this finding emphasises the significance of a precise and careful diagnosis: not all subjective sicca symptoms indicate the presence of pSS. In the few former studies in this context, the frequency of SS developing among patients with sicca symptoms has been even lower. In a prospective study among 45 patients with KCS complete SS was observed in only 17 % of the subjects (Forstot et al. 1982). In a long-term follow-up study, none of a total of 56 patients with isolated KCS was reported to have developed SS (Kruize et al. 1996). However, the follow-up assessment was not complete and did not include a second labial salivary gland biopsy, and only 67% of the KCS patients were reached for follow-up.

6.3.2 Ocular findings (I)

The frequency of subjective sicca symptoms in the eye increased and the objective ocular findings progressed over time in the SS group. The frequency of these subjective symptoms decreased over time in the sicca group and there was no progression in the objective ophthalmological findings in that group during the follow-up. The latter findings are in accordance with results of earlier studies (Kriegsbaum et al. 1988, Kruize et al. 1996).

6.3.3 Oral findings (I)

Similarly to the subjective sicca symptoms in the eyes, the frequency of such symptoms in the mouth increased over time in the SS group. However, subjective xerostomia symptoms remained comparable to the baseline situation in the sicca group.

There was a significant progression in the histological findings in labial salivary gland specimens over time in the present study. However, it must be remembered that since repeat biopsies were not performed on patients with diagnostic findings initially, no information on possible deterioration or healing of these lesions is available. In two former studies, serial assessments of histological findings have been described in patients with SS (Leroy et al 1992, Jonsson et al. 1993), but in no populations with sicca symptoms alone. In these two studies also, the lesions were mainly progressive with time.

6.3.4 Predictive factors for the development of SS (I)

High age at symptom onset was found to be a predictive factor for SS development. The effect of age on the presentation of SS has lately drawn attention and somewhat contradictory results have been reported. In pSS patients of young age at onset of disease, anti-SSA antibodies, positive RF and monoclonal immunoglobulins were encountered more frequently than in those old at onset (Ramos-Casals et al. 1998, Haga and Jonsson 1999). On the other hand, Garcia-Garrasco and associates (1999) found no notable differences in clinical and immunological characteristics in patients with pSS at older onset compared to others.

Positive ANA were found in the present study to be predictive of SS development in patients with sicca symptoms. In a long-term follow-up study involving pSS, sSS and KCS patients (Kruize et al. 1996) it was sought to identify laboratory factors predictive of the development of extraglandular symptoms of SS, but no such factors were found. The only predictive factors for SS development previously suggested have been anti-SSA and anti-SSB antibodies (Martinez-Lavin et al. 1979, Simmons-O'Brien et al. 1995). In the present study anti-SSA and anti-SSB antibodies were not available for the analysis of predictive factors. As here ANA positivity was found to be a factor predictive of SS development among patients with sicca symptoms, one might speculate that anti-SSA and anti-SSB antibodies, being more specific for SS, would presumably have gained even more significance in this respect.

In addition to positive ANA, high serum IgG concentrations were found in the present study to be predictive of SS development in patients with sicca symptoms.

Similar findings have been observed with regard to the course of established SS. Plasma level of IgG, serum level of ANA and assessment of focal sialadenitis in LSG were used as markers for immuno-inflammatory activity in a model suggested for classification of disease manifestations in pSS based on a retrospective, long-term observational clinical study (Asmussen et al. 1996). The baseline markers of disease activity were observed to be correlated with the long-term outcome of clinical disease (Asmussen et al. 1996).

Here, a high concentration of serum β_2m predicted SS development in patients with sicca symptoms. This is in agreement with a previous finding that higher mean serum concentrations of β_2m were present in patients with SS than in those with KCS only or controls (Ström et al. 1978). High serum β_2m levels have previously been reported especially in SS patients with lymphoproliferative complications or renal involvement (Michalski et al. 1975).

A small portion of the patients with initial sicca symptoms developed other rheumatic diseases by the time of the follow-up. Similarly, 39% of initially ANA and RF positive patients who were negative for anti-SSA and anti-SSB were given revised diagnoses over a follow-up period of 10 years (Davidson et al. 1999).

6.4 Cohort study of pSS patients (II)

6.4.1 General (II)

The cumulative clinical characteristics and the frequencies of extraglandular features (arthralgias and arthritis, Raynaud's symptom, purpura, chest symptoms, lymphadenopathy, serositis, renal and neurological symptoms) in the patients with pSS are in fair accord with findings hitherto (Pavlidis et al. 1982, Kelly et al. 1991, Markusse et al. 1992, Pease et al. 1993, Davidson et al. 1999).

In the course of time, mean blood ESR and the mean concentrations of serum IgG and IgM decreased in these patients with pSS, also in patients who had never been treated with either corticosteroids or DMARDs. This decrease is most probably not explained by increasing age, as an age-related increase in serum immunoglobulin IgA and IgG concentrations has been established in several studies of healthy populations (Riesen et al. 1976, Batory et al. 1984, Rajczy et al. 1986, Paganelli et al. 1992).

In an earlier follow-up study of SS patients (Kruize et al. 1996) including 21 with pSS, a decrease in mean serum IgG level was noted, as in the present study. However, unlike the present case, no statistically significant changes in the concentrations of blood ESR or serum IgM were noted during the follow-up. In patients with isolated KCS, ESR and IgA levels were higher at follow-up than at baseline.

In another follow-up study, improvement in the clinical features of pSS which could not be attributed to therapy occurred in 12 out of 100 patients (Kelly et al. 1991). This development was associated with a decrease in the titers of ANA or RF. Recently, Haga and Jonsson (1999) observed that young adult pSS patients had a higher frequency of auto-antibodies than middle-aged and elderly patients, indicating a more active immunological disease. As the presence of anti-SSA/SSB, RF and levels of serum IgG did not correlate negatively with disease duration from symptom onset, the authors concluded that the younger patients did not lose their immunological activity with age, but that there would be two entirely different subsets of pSS patients in respect of serological abnormalities.

Previously, a fall in levels of serum gammaglobulins, especially of IgM and autoantibodies, has been found to accompany or precede the onset of lymphoid malignancies in patients with SS (Talal and Bunim 1964, Whaley et al. 1973a). Since here the mean serum C3 concentrations in pSS patients were found to increase over time concomitant with the decline in serum IgG and IgM concentrations, these findings together might reflect diminishing inflammatory activity over time rather than developing lymphoid malignancies. These remarks are, however, purely speculative, as this cohort study did not evaluate whether the declining immunoglobulin concentrations were associated with any positive effects on the health of the patients. At least this decline did not eliminate the increased risk of lymphoma development in the cohort, a fact which supports previous suggestions of a monoclonal process initiated very early in the course of SS (Tzioufas et al. 1987).

6.4.2 Lymphoproliferation in pSS (I-II)

An excess risk of NHL in patients with SS was observed in this pSS patient cohort, in accord with a number of previous studies (Talal and Bunim 1964, Bloch et al. 1965, Anderson and Talal 1971, Kassan et al. 1978, McCurley et al. 1990, Kelly et al. 1991, Markusse et al. 1992, Pavlidis et al. 1992, Pease et al. 1993, Zufferey et al. 1995, Kruize et al. 1996, Kauppi et al. 1997, Davidson et al. 1999). The incidence of malignancies other than lymphoid was not increased in this patient cohort, as also observed in the epidemiological study by Kassan and colleagues (1978).

Three of the 110 pSS patients in this study developed NHL. Most lymphomas complicating the course of SS are derived from B-lymphocytes (Shin et al. 1991). One of the NHLs in these patients was an ileal T-cell lymphoma, a form unusual in SS (Wilke et al. 1984, Isenberg et al. 1987, Schuurman et al. 1987, Rustin et al. 1988, van der Valk et al. 1989).

However, no cases of lymphoma were recorded among the patients with sicca symptoms. This might be explained by a shorter observation period from symptom onset compared to a population where the diagnosis of SS is already apparent. However, in the epidemiological study by Kassan and associates (1978) the risk of lymphoma in SS was not associated with either age or duration of disease. Ramos-Casals and colleagues (1998) report a higher incidence of lymphomas in patients with pSS with symptom onset at young as against old age. Nor did any patients with isolated KCS develop lymphoproliferative disease during the follow-up in two former studies (Zufferey et al. 1995, Kruize et al. 1996).

6.4.3 Risk factors for NHL (II)

The patients who developed lymphoma evinced several extraglandular features of SS. Regarding the clinical presentation at baseline, however, no statistically significant differences could be observed at least in the glandular findings compared to other patients. Usually, SS patients who develop lymphoid neoplasms have had a severe sicca syndrome with many of its complications (Talal et al. 1967, Sutcliffe et al. 1998). These clinical observations could not be confirmed by McCurley and associates (1990): age, duration of disease, extent of lymphadenopathy, splenomegaly, or parotid swelling did not identify the patients at risk of lymphoma. In a recent European multicenter study lymphadenopathy, skin vasculitis, peripheral nerve involvement, low-grade fever, anemia and lymphopenia were observed more frequently in SS patients with NHL than in those without (Voulgarelis et al. 1999). Purpura, decreased C4 complement levels and mixed cryoglobulinemia, were found to be adverse prognostic factors in a recent large cohort study (Skopouli et al. 2000)

The pSS patients with subsequent lymphoma here had higher baseline IgA, IgM and β_2m levels than those without. High serum β_2m was found to be an independent predictive factor for lymphoma development in pSS patients. The number of patients with malignant lymphoproliferation was small in this study, and thus caution is warranted regarding results identifying patients at risk of developing NHL, although the results in this respect were parallel to previous findings. Elevated levels of serum β_2m have previously been associated with renal and lymphoproliferative complications (Michalski et al. 1975) of SS and pulmonary hyperinflation in patients with pSS (Lahdensuo and Korpela 1995)

6.4.4 Mortality in SS (II)

The standardised mortality ratio for female pSS patients in this cohort was only slightly increased compared to that in the general population. Recently an increased SMR (2.07) was reported in a cohort of 261 pSS patients with a median follow-up time of three years (Skopouli et al. 2000). Hitherto, only one population-based cohort study on survivorship in SS has been published; in that study patients with pSS were not found to have increased mortality (Martens et al. 1999).

6.5 Renal involvement in pSS (III–IV)

6.5.1 The occurrence of dRTA and proteinuria (III)

Representing the largest material of pSS patients with examinations of renal acidification capacity, these results confirm the frequency of dRTA in pSS to lie around 30%. This result was based strictly on findings in pSS patients and is in fair accord with most previous reports (Shearn and Tu 1965, Talal et al. 1968, Whaley et al. 1973a, Siamopoulos et al. 1986, Shiozawa et al. 1987, Pokorny et al. 1989) but is higher than figures reported by Viergever and Swaak (1991) (12%) and Vitali and colleagues (1991) (15%) but lower than those given by Eriksson and associates (1995) (67%).

Proteinuria in pSS patients was detected more frequently than in earlier reports, but in keeping with previous findings the quantity of proteinuria was of minor degree. However, this is hitherto the largest material of pSS patients where urinary protein excretion has been systematically investigated.

Increased urinary α_1m excretion reflects particularly proximal rather than distal tubular disorders (Yu et al. 1983). Signs of tubular proteinuria, detected by urinary α_1m, were found here relatively rarely (in 12% of the patients). Excretion of urinary α_1m as a sign of tubular proteinuria has previously been used in only one study of pSS patients (Eriksson et al 1995), where increased excretion was observed in up to 46 % of the group.

In spite of the instability of urinary β_2m at low pH, higher figures for tubular proteinuria than in the present study have been reported with increased urinary β_2m excretion as an indicator of proximal tubular dysfunction. Shiozawa and associates (1987) found increased urinary β_2m excretion in four out of 11 patients (36%) and Viergever and Swaak (1991) in seven of their 27 pSS patients (26%).

6.5.2 The occurrence of renal impairment (III)

Mild azotemia is possible in patients with pSS (Tu et al. 1968). Although overt or latent dRTA was a frequent finding in pSS patients in this study, frank renal impairment in association with renal tubular acidification defect seldom occurred, similarly to findings by Vitali and colleagues (1991). All except two of the subjects with increased serum creatinine levels and all of those with decreased creatinine clearance were aged over 65 years. Similarly to previous studies the renal impairment observed in our pSS patients was only of mild to moderate degree (Eriksson et al. 1995).

6.5.3 Factors associated with the occurrence of dRTA (III)

The patients with latent or overt dRTA did not differ in mean age from the other patients in the present study, similarly to observations by Shioji and associates (1970). In two previous studies (Shiozawa et al. 1987, Pokorny et al. 1989) the patients with dRTA were younger than those with normal renal tubular acidification capacity. The duration of the disease, and especially that of xerostomia, was significantly longer in patients with latent or overt dRTA than in the others. Similar (Shiozawa et al. 1987) and contrasting (Pokorny et al. 1989) findings have been reported.

Hypertension and mild proteinuria occurred more frequently in pSS patients with dRTA than in those with normal urinary acidification capacity. Similar observations have not previously been reported.

Serum β_2m concentration was significantly higher in patients with latent or overt dRTA than in those with normal acidification capacity. A high level of serum β_2m has also previously been associated with renal findings in SS (Michalski et al. 1975). Among 97 SS patients, serum β_2m exceeded 4 μg/ml in 85% of those with associated renal disease (RTA or lupus nephritis), but in only 14% of those without either renal or lymphoproliferative complications (Michalski et al. 1975).

The serum concentrations of β_2m and creatinine are closely correlated in subjects with elevated serum levels of creatinine (Wibell et al. 1973). The serum level of β_2m is determined both by the rate of synthesis and by the glomerular filtration rate (Karlsson et al. 1980). Increased production of serum β_2m is observed in conditions involving neoplastic proliferation of lymphoid B-cells or in inflammatory disorders connected with an activation of the lymphopoetic system (Karlsson et al. 1980). Thus both increased production of the protein as well as reduced GFR may account for raised serum β_2m concentrations in pSS patients with dRTA.

In the present study no significant differences in serum IgG concentrations were found between patients with dRTA and those with normal acidification capacity, although there was a tendency for serum IgG levels to be higher in the dRTA group. Previously, hypergammaglobulinemia has been associated with dRTA both in SS and in other conditions (Shearn and Tu 1965, McCurdy et al. 1967, Morris and Fudenberg 1967, Talal et al. 1968). On the other hand, no significant associations between serum gammaglobulin concentration and dRTA were found in a number of studies (Pokorny et al. 1989, Viergever and Swaak 1991, Vitali et al. 1991, Siamopoulos et al. 1992).

In the present series no differences emerged in the occurrence of anti-SSA or anti-SSB antibodies between patients with dRTA and normal acidification capacity. Similar findings regarding their association with renal abnormalities have been reported elsewhere (Pokorny et al. 1989, Siamopoulos et al. 1992), although anti-SSA and anti-SSB antibodies have previously been associated particularly with extraglandular manifestations of SS (Moutsopoulos and Zerva 1990).

6.5.4 Factors associated with the occurrence of proteinuria (III)

There was no significant difference between the mean age of the patients with proteinuria and those with normal urinary protein excretion. The duration of xerostomia was longer, the levels of systolic and diastolic blood pressure were higher, dRTA occurred more frequently and the levels of serum β_2m were higher in pSS patients with proteinuria compared to those without. Furthermore, it was noteworthy that in the subgroup of pSS patients whose acidification capacity was evaluated, the presence of proteinuria was in a logistic regression model significantly associated with RTA. Thus in patients with pSS, mild proteinuria might indirectly imply the possibility of a defect in renal acidification capacity. However, the clinical significance of these findings must of necessity be confirmed, as no such comparisons in respect of proteinuria in pSS have previously been presented.

6.5.5 Predictive factors for renal involvement (IV)

High serum total protein and gammaglobulin levels were here risk factors underlying later development of dRTA, consistently with the report of Talal and colleagues (1968). In addition, high levels of serum β_2m were associated with the future development of dRTA, mild proteinuria and increased urinary excretion of α_1m in pSS patients.

Michalski and co-workers (1975) have previously shown an association of high serum β_2m levels with clinically obvious renal manifestations in SS.

Previously, even a causative effect of hypergammaglobulinemia for the development of RTA has been suggested (Morris and Fudenberg 1967). However, renal interstitial lymphocytic infiltrates were demonstrated in three out of four SS patients with dRTA and in none of four patients with normal renal acidification capacity, despite the presence of hypergammaglobulinemia in both groups (Shioji et al. 1970). Mere hypergammaglobulinemia does not cause dRTA, as dRTA was not observed in RA patients when studied with ammonium chloride loading tests, even if they were hypergammaglobulinemic (Pasternack et al. 1970).

An immunological mechanism of the renal tubular impairment in SS has been suggested by histological studies (Talal et al. 1968, Pasternack and Linder 1970, Shioji et al. 1970). The association of a high concentration of serum β_2m with latent or overt dRTA also implies that the renal manifestation may be a consequence of prolonged active lymphoproliferation.

Positivity in ANA did not predict the development of subsequent latent or overt distal RTA, proteinuria or increased excretion of urinary α_1m. However, as baseline ENA antibody determinations were not comprehensively available in these patients, the possible role of the more disease-specific anti-SSA and anti-SSB antibodies as predictive factors for renal involvement could not be evaluated. However, in most previous studies, pSS patients with anti-SSA antibodies have been found to be more prone to develop systemic disease (Kelly et al. 1991, Pease et al. 1993). In one follow-up study, however, no significant differences were found between patients with and without renal manifestations in the occurrence either of RF, ANA, anti-SSA and anti-SSB or of anti-DNA antibodies (Pokorny et al. 1989).

As the renal acidification defect in the vast majority of these patients was only subclinical, the precise time-point at which these defects emerged is not known. Definite exclusion of patients with possible subclinical renal manifestations at the onset would have necessitated ammonium chloride loading tests also at baseline. This limitation has to be remembered when interpreting the results, especially as the degree of statistical significance of most factors found to be predictive of renal involvement was not very strong.

6.6 Clinical associations of IL-6 plasma levels and promoter region gene polymorphism in pSS (V)

The IL-6 plasma levels were increased in patients with pSS compared to healthy controls, similarly to findings originally reported by Pettersson and colleagues (1992). However, this was the first time that high IL-6 levels were shown to be associated with clinical features and specific extraglandular manifestations of pSS.

Plasma IL-6 levels increased in parallel with the histological grade in LSG biopsies as well as the number of pSS criteria fulfilled. The lymphocyte infiltration grade of LSG histology is one of the most specific diagnostic markers of pSS, and the number of pSS criteria fulfilled can also be considered to reflect the severity of pSS.

Local and systemic increases in IL-6 and other pro-inflammatory cytokines have been observed in association with gluten sensitivity (Przemioslo et al. 1994, Fornari et al. 1998) and could explain the elevated IL-6 levels in pSS patients with celiac disease.

IL-6 levels were higher in pSS patients with lung fibrosis or alveolitis than in those without. The diagnosis of pulmonary fibrosis was based on chest radiograph examinations only, not on HRCT or diffusion capacity determinations. However, the finding seems relevant, as IL-6 has previously been suggested also to play a role in the development of both diffuse interstitial fibrosis (Shahar et al. 1996) and pulmonary fibrosis in systemic sclerosis (Hasegawa et al. 1998).

The etiology of peripheral neuropathies in SS is held to be related to inflammatory vascular disease of the vasa nervorum (Kaltreider and Talal 1969, Alexander et al. 1981). The possible role of plasma IL-6 levels in the pathogenesis of the PNS symptoms in pSS patients remains to be ascertained. Identification of PNS symptoms relied on patient records and interview, not on systematic neurological examinations, which limits judgement of the significance of the findings.

The G/C polymorphism of the IL-6 gene does not predispose to pSS, as the IL-6 allele frequencies were similar in patients and normal subjects. However, the plasma IL-6 levels were regulated by the IL-6 genotype in pSS patients but not in controls. Furthermore, the circulating IL-6 was related to specific manifestations of pSS. Thus, allele G carrier status and the high IL-6 levels associated with it could be related to the severity of pSS and could constitute risk factors for certain extraglandular manifestations associated with pSS.

7 Summary and conclusions

In the present studies the long-term outcome of patients with sicca symptoms (I) and of patients with pSS (II) was assessed. The occurrence of renal involvement in pSS (III) and the factors predictive of it (IV), were investigated. Further, IL-6 plasma levels and the gene polymorphism of IL-6 and their possible association with clinical features of pSS were examined (V).

Thirty-one of the 87 subjects (36%) with symptoms of dryness of either eyes or mouth, developed SS after a median follow-up time of 11 years (I). No cases of lymphoma were found during the follow-up of patients with sicca symptoms. Factors which could predict the development of clinical SS in patients with sicca symptoms were: high age, positive ANA, elevated levels of serum gammaglobulin and serum IgG as well as a high level of serum β_2m.

Factors identified as suggestive for a diagnosis of pSS among patients with sicca symptoms might aid in recognising this condition in clinical work. On the other hand, the fact that only a third of patients with subjective sicca symptoms developed SS during follow-up underlines the value of objective findings and careful and precise diagnostic procedures.

In a cohort of 110 patients with pSS levels of serum IgM and IgG were found to decline in the course of time (II). This tendency was not explained by treatment. As in previous studies, the risk of NHL in this cohort of pSS patients was increased (II). The pSS patients subsequently developing lymphoma had higher baseline serum β_2m levels than the others. The small number of NHL cases in this cohort limits interpretation of the result. However, the clinician should keep in mind the possibility of subsequent malignant lymphoproliferation particularly in pSS patients with elevated baseline serum β_2m concentrations.

The present study (III) is the largest series published hitherto in which the occurrence of renal manifestations in pSS has been investigated. Mild proteinuria was found in up to 44% of the 78 pSS patients, which is more frequently than previously reported. Similarly to earlier findings, clinically significant proteinuria appeared infrequently. Long duration of xerostomia, high systolic and diastolic blood pressure and high level of serum β_2m were found more often in patients with proteinuria as compared with others. The associations of the occurrence of proteinuria with clinical features of pSS have not previously been reported. Therefore, the clinical significance of the findings needs to be ascertained.

The presence of dRTA was investigated by ammonium chloride loading tests in 55 pSS patients (III). The frequency of latent or overt dRTA was confirmed to lie around 30%. Duration of disease, and especially of xerostomia, was significantly longer in patients with dRTA than in those with normal acidification capacity. Hypertension, mild proteinuria and raised levels of serum β_2m occurred more frequently in pSS patients with dRTA. Renal impairment occurred seldom in association with dRTA. As the frequency of overt RTA was low, there seems to be no need for routine ammonium chloride loading tests in pSS patients in clinical practice. To recognise the frequently encountered latent dRTA, concomitant analysis of acid-base balance and of the urinary pH should be considered at least in the presence of suggestive clinical findings.

An elevated baseline serum β_2m level was found to predict subsequent clinical and subclinical renal findings (dRTA, proteinuria, urinary α_1m excretion) in pSS patients (IV). Other predictors of subsequent dRTA in pSS patients were elevated baseline serum gammaglobulin, serum IgG and serum protein levels. Serum β_2m might be a useful parameter in clinical practice in the follow-up of pSS patients – elevated levels should warrant alertness particularly to possible renal or lymphoproliferative complications of pSS.

IL-6 plasma levels and the genetic polymorphism of IL-6 was investigated in 66 pSS patients and in healthy controls (V). The IL-6 gene allele frequencies in the respective groups were similar. The IL-6 plasma levels were genetically regulated in pSS patients but not in healthy controls. IL-6 was found to be associated with specific clinical manifestations of pSS such as the histological grade in LSG biopsies, associated celiac disease, pulmonary fibrosis or PNS symptoms.

Acknowledgements

This study was carried out at the Department of Internal Medicine, Tampere University Hospital during the years 1994–2000.

First I wish to express my deepest gratitude and sincere admiration to my supervisor Professor Amos Pasternack, M.D., Head of the Department of Medicine, Tampere University Hospital. It has been a great privilege for me to have had the opportunity to enjoy his lectures while still studying medicine, to specialise in internal medicine and later on to continue working in the clinic led by him, and finally to start and complete this thesis under his wise, stimulating and encouraging guidance.

I wish to honour the memory of Docent Anni Vilppula, M.D., former Head of the Department of Rheumatology. Her interest in Sjögren's syndrome arose already in the 1970s and the present work would not have been possible without her visions from those times. As a young doctor I had the opportunity to work under her demanding, but just and enthusiastic influence. The friendship that arose then gave me the possibility to share with her the joy of the awareness of this work coming to completion.

The official reviewers of this thesis were Professor Yrjö T. Konttinen, M.D. and Docent Marianne Gripenberg-Gahmberg, M.D. I wish to thank them for their constructive criticism and most valuable comments on the manuscript.

I am particularly obliged to Docent Markku Korpela, M.D., for his significant contribution to the planning of the study and for the patient guidance and accurate instructions on scientific work he has provided me. I wish to express my profound gratitude to him.

I am most grateful to my co-authors Professor Heikki Helin, M.D., Professor Mikko Hurme, M.D., Professor Juhani Pukander, M.D., Docent Timo Kouri, M.D., Docent Ari Miettinen, M.D., Docent Eero Pukkala, Ph.D., Docent Hannu Uusitalo, M.D., Jaakko Antonen, M.D., Ph.D. and Janne Hulkkonen, M.D. for excellent and rewarding collaboration during this study. This work would never have been possible without their expert contribution.

I am greatly indebted to Professor Pekka Laippala, Ph.D. for his expert advice in the field of statistics and for his most encouraging attitude. The help and advice of Anna-Maija Koivisto, B.Sc. in the statistical work is thankfully acknowledged. I am also obliged to Tiina Salminen, Ph.D. for her assistance.

I sincerely thank Professor Jukka Mustonen, M.D., member of the follow-up group of this thesis, for useful advice and comments on the manuscript. I also warmly

thank Urpo Yli-Kerttula, M.D., Ph.D., Head of the Department of Rheumatology, for positive and understanding attitude towards my work.

I am truly grateful to the entire personnel of the Department of Rheumatology and the Rheumatological Outpatient Clinic for their practical help. The excellent assistance of Ms. Airi Lappalainen and Ms. Heidi Mäkinen in organising the examinations of patients has been invaluable. I wish to extend my warmest thanks to the staff of the Department of Clinical Physiology for their co-operation. My thanks are due particularly to Ms. Maija Inkiläinen, Ms. Taina Soro-Piiroinen and Ms. Marjo Airut.

I am most thankful to Mr. Robert MacGilleon, M.A. for careful and swift revision of the English language of this thesis.

I appreciate the skilful secretarial work of Ms. Marita Hallila.

I most sincerely thank all the patients who took part in this study for their admirable co-operation.

Most of all, I thank my family and friends for their invaluable support and encouragement.

I am grateful for the financial support provided for this study by the Medical Research Fund of Tampere University Hospital, the Maud Kuistila Foundation, the Finnish Kidney Foundation, the University of Tampere, the City of Tampere and the Tampere Rheumatism Association.

Tampere, May 2001

Marja Pertovaara

References

Adler S, Lindeman RD, Yiengst MJ, Beard E and Shock NW (1968): Effect of acute acid loading on urinary acid excretion by the aging human kidney. J Lab Clin Med 72: 278–289.

Alexander EL (1987): Inflammatory vascular disease in Sjögren's syndrome. In: Sjögren's syndrome. Clinical and immunological aspects, pp. 102–125. Eds. N Talal, HM Moutsopoulos and SS Kassan, Springer-Verlag, Berlin.

Alexander EL, Provost TT, Stevens MB and Alexander GE (1982a): Neurologic complications of primary Sjögren's syndrome. Medicine (Baltimore) 61: 247–257.

Alexander EL, Hirsch TJ, Arnett FC, Provost TT and Stevens MB (1982b): Ro(SSA) and La (SSB) antibodies in the clinical spectrum of Sjögren's syndrome. J Rheumatol 9: 239–246.

Alexander EL, Arnett FC, Provost TT and Stevens MB (1983): Sjögren's syndrome: association of anti-Ro(SS-A) antibodies with vasculitis, hematologic abnormalities, and serologic hyperreactivity. Ann Intern Med 98: 155–159.

Alexander GE, Provost TT, Stevens MB and Alexander EL (1981): Sjögren's syndrome. Central nervous system manifestations. Neurology 31: 1391–1396.

Alspaugh MA and Tan EM (1975): Antibodies to cellular antigens in Sjögren's syndrome. J Clin Invest 55: 1067–1073.

Anderson LG and Talal N (1971): The spectrum of benign to malignant lymphoproliferation in Sjögren's syndrome. Clin Exp Immunol 9: 199–221.

Andonopoulos AP and Ballas C (1995): Autonomic cardiovascular neuropathy in primary Sjögren's syndrome. Rheumatol Int 15: 127–129.

Andonopoulos AP, Skopouli FN, Dimou GS, Drosos AA and Moutsopoulos HM (1990): Sjögren's syndrome in systemic lupus erythematosus. J Rheumatol 17: 201–204.

Antonen JA, Markula KP, Pertovaara MI and Pasternack AI (1999): Adverse drug reactions in Sjögren's syndrome; frequent allergic reactions and a specific trimethoprim-associated systemic reaction. Scand J Rheumatol 28: 157–159.

Asmussen K, Andersen V, Bendixen G, Schiodt M and Oxholm P (1996): A new model for classification of disease manifestations in primary Sjögren's syndrome: evaluation in a retrospective long-term study. J Intern Med 239: 475–482.

Backman U, Danielsson B and Sohtell M (1976): A short duration renal acidification test. Scand J Urol Nephrol Suppl 35: 33–47.

Bacman S, Perez Leiros C, Sterin-Borda L, Hubscher O, Arana R and Borda E (1998): Autoantibodies against lacrimal gland M3 muscarinic acetylcholine receptors in patients with primary Sjögren's syndrome. Invest Ophthalmol Vis Sci 39: 151–156.

Barendregt PJ, Visser MRM, Smets EMA, Tulen JHM, van den Meiracker AH, Boomsma F and Markusse HM (1998): Fatigue in primary Sjögren's syndrome. Ann Rheum Dis 57: 291–295.

Bastani B, Haragsim L, Gluck S and Siamopoulos KC (1995): Lack of H-ATPase in distal nephron causing hypokalaemic distal RTA in a patient with Sjögren's syndrome. Nephrol Dial Transplant 10: 908–913.

Batory G, Jancso A, Puskas E, Redei A and Lengyel E (1984): Antibody and immunoglobulin levels in aged humans. Arch Gerontol Geriatr 3: 175–188.

Bloch K, Buchanan W, Wohl M and Bunim J (1965): Sjögren`s syndrome: a clinical, pathological and serological study of sixty-two cases. Medicine (Baltimore) 44: 187–231.

Bodeutsch C, de Wilde PC, Kater L, van den Hoogen FH, Hene RJ, van Houwelingen JC, van de Putte LB and Vooijs GP (1993): Monotypic plasma cells in labial salivary glands of patients with Sjögren's syndrome: prognosticator for systemic lymphoproliferative disease. J Clin Pathol 46: 123–128.

Bonafede RP, Downey DC and Bennett RM (1995): An association of fibromyalgia with primary Sjögren's syndrome: a prospective study of 72 patients. J Rheumatol 22: 133–136.

Calabrese LH, Davis ME and Wilke WS (1994): Chronic fatigue syndrome and a disorder resembling Sjögren's syndrome: preliminary report. Clin Infect Dis 18 Suppl 1: S 28–31.

Castro-Poltronieri A and Alarcon-Segovia D (1983): Articular manifestations of primary Sjögren's syndrome. J Rheumatol 10: 485–488.

Chanana A and Sharma OP (1997): Pulmonary involvement in unusual multisystem disorders. Curr Opin Pulm Med 3: 384–390.

Chisholm DM and Mason DK (1968): Labial salivary gland biopsy in Sjögren's syndrome. J Clin Pathol 21: 656–660.

Cohen EP, Bastani B, Cohen MR, Kolber S, Hemken P and Gluck SL (1992): Absence of H^+-ATPase in cortical collecting tubules of a patient with Sjögren's syndrome and distal renal tubular acidosis. J Am Soc Nephrol 3: 264–271.

Constantopoulos S, Papadimitriou C and Moutsopoulos H (1985): Respiratory manifestations in primary Sjögren's syndrome. Chest 88: 226–229.

Dabadghao S, Aggarwal A, Arora P, Pandey R and Misra R (1995): Glomerulonephritis leading to end stage renal disease in a patient with primary Sjögren's syndrome. Clin Exp Rheumatol 13: 509–511.

Daniels TE and Whitcher JP (1995): On oral lymphocytic sialadenitis as a gold standard for the diagnosis of Sjögren's syndrome: comment on the article by Daniels and Whitcher. Reply. Arthritis Rheum 38: 870–872.

Davidson BK, Kelly CA and Griffiths ID (1999): Primary Sjögren's syndrome in the north east of England: a long-term follow-up study. Rheumatology 38: 245–253.

DeFranco PE, Haragsim L, Schmitz PG, Bastani B and Li JP (1995): Absence of vacuolar H^{+-}ATPase pump in the collecting duct of a patient with hypokalemic distal renal tubular acidosis and Sjögren's syndrome. J Am Soc Nephrol 6: 295–301.

Dowd JE and Lipsky PE (1993): Sjögren's syndrome presenting as hypokalemic periodic paralysis. Arthritis Rheum 36: 1735–1738.

Drosos AA, Andonopoulos AP, Costopoulos JS, Stavropoulos ED, Papadimitriou CS and Moutsopoulos HM (1988): Sjögren's syndrome in progressive systemic sclerosis. J Rheumatol 15: 965–968.

Drosos AA, Constantopoulos SH, Psychos D, Stefanou D, Papadimitriou CS and Moutsopoulos HM (1989): The forgotten cause of sicca complex; sarcoidosis. J Rheumatol 16: 1548–1551.

Dubost JJ, Perrier S, Afane M, Viallard JL, Roux-Lombard P, Baudet-Pommel M, Begue C, Kemeny JL and Sauvezie B (1996): IL-1 receptor antagonist in saliva; characterization in normal saliva and reduced concentration in Sjögren's syndrome (SS). Clin Exp Immunol 106: 237–242.

Eneström S, Denneberg T and Eriksson P (1995): Histopathology of renal biopsies with correlation to clinical findings in primary Sjögren's syndrome. Clin Exp Rheumatol 13; 697–703.

Eriksson P, Denneberg T, Larsson L and Lindström F (1995): Biochemical markers of renal disease in primary Sjögren's syndrome. Scand J Urol Nephrol 29: 383–392.

Eriksson P, Denneberg T, Eneström S, Johansson B, Lindström F and Skogh T (1996a): Urolithiasis and distal renal tubular acidosis preceding primary Sjögren's syndrome: a retrospective study 5–53 years after the presentation of urolithiasis. J Intern Med 239: 483–488.

Eriksson P, Denneberg T, Granerus G and Lindström F (1996b): Glomerular filtration rate in primary Sjögren's syndrome with renal disease. Scand J Urol Nephrol 30: 121–127.

Eriksson P, Denneberg T, Lundström I, Skogh T and Tiselius H-G (1997): Autoantibodies and primary Sjögren's syndrome in a hypocitraturic stone population. Scand J Urol Nephrol 31: 73–80.

Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S and Woo P (1998): The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 102: 1369–1376.

Font J, Cervera R, Lopez-Soto A, Darnell A and Ingelmo M (1989): Mixed membranous and proliferative glomerulonephritis in primary Sjögren's syndrome. Br J Rheumatol 28: 548–550.

Fornari MC, Pedreira S, Niveloni S, Gonzalez D, Diez RA, Vazquez H, Mazure R, Sugai E, Smecuol E, Boerr L, Maurino E and Bai JC (1998): Pre- and post-treatment serum levels of cytokines IL-1beta, IL-6, and IL-1 receptor antagonist in celiac disease. Are they related to the associated osteopenia? Am J Gastroenterol 93: 413–418.

Forstot JZ, Forstot SL, Greer RO and Tan EM (1982): The incidence of Sjögren's sicca complex in a population of patients with keratoconjunctivitis sicca. Arthritis Rheum 25: 156–160.

Fox R, Robinson C, Curd J, Kozin F and Howell F (1986): Sjögren's syndrome. Proposed criteria for classification. Arthritis Rheum 29: 577–585.

Fox RI, Törnwall J, Maruyama T and Stern M (1998): Evolving concepts of diagnosis, pathogenesis, and therapy of Sjögren's syndrome. Curr Opin Rheumatol 10: 737–740.

Gannot G, Lancaster HE and Fox PC (2000): Clinical course of primary Sjögren's syndrome: salivary, oral and serologic aspects. J Rheumatol 27; 1905–1909.

Garcia-Carrasco M, Ramos M and Cervera R (1997): Hepatitis C virus infection in primary Sjögren's syndrome: prevalence and clinical significance in a series of 90 patients. Ann Rheum Dis 56: 173–175.

Garcia-Carrasco M, Cervera R, Rosas J, Ramos-Casals M, Morla RM, Siso A, Jimenez S, Pallares L, Font J and Ingelmo M (1999): Primary Sjögren's syndrome in the elderly: clinical and immunological characteristics. Lupus 8: 20–23.

Gentric A, Herve JP, Pennec YL, Leroy JP and Cledes J (1989): Severe renal involvement in primary Sjögren's syndrome. Adv Exp Med Biol 252: 73–81.

Gerhardt RE, Loebl DH and Rao RN (1978): Interstitial immunofluoresence in nephritis of Sjögren's syndrome. Clin Nephrol 10: 201–207.

Gogel HK, Searles RB, Volpicelli NA and Cornwell GG (1983): Primary amyloidosis presenting as Sjögren's syndrome. Arch Intern Med 143: 2325–2326.

Goldman JA and Julian EH (1977): Pseudo-Sjögren syndrome with hyperlipoproteinemia. JAMA 237: 1582–1584.

Goules A, Masouridi S, Tzioufas AG, Ioannidis JP, Skopouli FN and Moutsopoulos HM (2000): Clinically significant and biopsy-documented renal involvement in primary Sjögren's syndrome. Medicine (Baltimore) 79: 241–249.

Greenspan JS, Daniels TE, Talal N and Sylvester RA (1974): The histopathology of Sjögren's syndrome in labial salivary gland biopsies. Oral Surg 37: 217–229.

Griffith KJ, Chan EK, Lung CC, Hamel JC, Guo X, Miyachi K and Fritzler MJ (1997): Molecular cloning of a novel 97-kd Golgi complex autoantigen associated with Sjögren's syndrome. Arthritis Rheum 40: 1693–1702.

Grisius MM, Bermudez DK and Fox PC (1997): Salivary and serum interleukin 6 in primary Sjögren's syndrome. J Rheumatol 24: 1089–1091.

Haga H-J and Jonsson R (1999): The influence of age on disease manifestations and serological characteristics in primary Sjögren's syndrome. Scand J Rheumatol 28: 227–232.

Hagiwara E, Pando J, Ishigatsubo Y and Klinman DM (1998): Altered frequency of type 1 cytokine secreting cells in the peripheral blood of patients with primary Sjögren's syndrome. J Rheumatol 25: 89–93.

Hakala M and Niemelä RK (2000): Does autonomic nervous impairment have a role in pathophysiology of Sjögren's syndrome? Lancet 355: 1032–1033.

Halse A, Tengner P, Wahren-Herlenius M, Haga H and Jonsson R (1999): Increased frequency of cells secreting interleukin-6 and interleukin-10 in peripheral blood of patients with primary Sjögren's syndrome. Scand J Immunol 49: 533–538.

Han ZC, Bellucci S and Caen JP (1991): Megakaryocytopoiesis: characterization and regulation in normal and pathologic states. Int J Hematol 54: 3–14.

Haneji N, Nakamura T, Takio K, Yanagi K, Higashiyama H, Saito I, Noji S, Sugino H and Hayashi Y (1997): Identification of alpha-fodrin as a candidate autoantigen in primary Sjögren's syndrome. Science 276: 604–607.

Hardin JA (1975): Case records of the Massachusetts General Hospital 2292: 1285–1290.

Hasegawa M, Sato S, Fujimoto M, Ihn H, Kikuchi K and Takehara K (1998): Serum levels of interleukin 6 (IL-6), oncostatin M, soluble IL-6 receptor, and soluble gp130 in patients with systemic sclerosis. J Rheumatol 25: 308–313.

Hietaharju A, Yli-Kerttula U, Häkkinen V and Frey H (1990): Nervous system manifestations in Sjögren's syndrome. Acta Neurol Scand 81: 144–152.

Hochberg MC, Tielsch J, Munoz B, Bandeen-Roche K, West SK and Schein OD (1998): Prevalence of symptoms of dry mouth and their relationship to saliva production in community dwelling elderly: the SEE project. Salisbury eye evaluation. J Rheumatol 25: 486–491.

Homma M, Tojo T, Akizuki M and Yagamata H (1986): Criteria for Sjögren's sndrome in Japan. Scand J Rheumatol Suppl 61: 26–27.

Hradsky M, Hybasek I, Cenoch V, Sazmova V and Juran J (1967a): Oesophageal abnormalities in Sjögren's syndrome. Scand J Gastroenterol 2: 200–203.

Hradsky M, Bartos V and Keller O (1967b): Pancreatic function in Sjögren's syndrome. Gastroenterologia 108: 252–260.

Ichikawa Y, Takaya M, Uchiyama M, Shimizu H, Tokunaga M, Saitoh H and Arimori S (1988): Prospective studies on the progress of sicca symptoms in Sjögren's syndrome: subclinical disease develops into the clinical syndrome. Tokai J Exp Clin Med 13: 85–90.

Iltanen S, Collin P, Korpela M, Holm K, Partanen J, Polvi A and Mäki M (1999): Celiac disease and markers of celiac disease latency in patients with primary Sjögren's syndrome. Am J Gastroenterol 94: 1042–1046.

Isenberg DA, Hammond L, Fisher C, Griffiths M, Stewart J and Bottazzo GF (1982): Predictive value of SS-B precipitating antibodies in Sjögren's syndrome. BMJ 284: 1738–1740.

Isenberg DA, Griffiths MH, Rustin M, Webb MWS and Souhami RL (1987): T cell lymphoma in a patient with longstanding rheumatoid arthritis and Sjögren's syndrome. Arthritis Rheum 30: 115–117.

Jakobsson LTH, Axell TE, Hansen BU, Henricsson VJ, Larsson Å, Lieberkind K, Lilja B and Manthorpe R (1989): Dry eyes or mouth-an epidemiological study in Swedish adults, with special reference to primary Sjögren's syndrome. J Autoimmun 2:521–527.

Janin A, Morel P, Quiquandon I, Farre I, Hatron PY, Bauters F and Gosselin B (1992): Non-Hodgkin's lymphoma and Sjögren's syndrome: an immunopathological study of 113 patients. Clin Exp Rheumatol 10: 565–570.

Jonsson R, Kroneld U, Bäckman K, Magnusson B and Tarkowski A (1993): Progression of sialadenitis in Sjögren's syndrome. Br J Rheumatol 32: 578–581.

Jonsson R, Ohlsson M, Iakimtchouk K and Brokstad K (1999): Sjögren's syndrome-etiopathogenesis, apoptosis and animal models. In: The 100-year anniversary of Henrik Sjögren. State of the art lectures on Sjögren's syndrome and historical perpectives, pp. 68–73. Eds. P Eriksson and R Jonsson, Hygiea, Jönköping.

Jonsson R, Haga HJ and Gordon TP (2000): Current concepts on diagnosis, autoantibodies and therapy in Sjögren's syndrome. Scand J Rheumatol 29: 341–348.

Jordan R and Speight P (1996): Lymphoma in Sjögren's syndrome: From histopathology to molecular pathology. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 81: 308–320.

Jordan SC, Sakai R, Tabak MA, Ettenger RB, Cohen AH and Fine RN (1985): Induction of renal tubular dysfunction by transplacentally acquired IgG from a mother with Sjögren's syndrome. J Pediatr 107: 566–569.

Julkunen H, Kurki P, Kaaja R, Heikkilä R, Immonen I, Chan EKL, Wallgren E and Friman C (1993): Isolated congenital heart block. Arthritis Rheum 36: 1588–1598.

Kaltreider HB and Talal N (1969): The neuropathy of Sjögren's syndrome. Trigeminal nerve involvement. Ann Intern Med 70: 751–762.

Karlsson FA, Wibell L and Evrin PE (1980): Beta 2-microglobulin in clinical medicine. Scand J Clin Lab Invest Suppl 154: 27–37.

Karsh J, Pavlidis N, Weintraub BD and Moutsopoulos HM (1980): Thyroid disease in Sjögren's syndrome. Arthritis Rheum 23: 1326–1329.

Kassan SS and Gardy M (1978): Sjögren's syndrome: an update and overview. Am J Med 64: 1037–1046.

Kassan SS and Talal N (1987): Renal disease with Sjögren's syndrome. In: Sjögren's syndrome. Clinical and immunological aspects, pp. 96–101. Eds. N Talal, HM Moutsopoulos and SS Kassan, Springer-Verlag, Berlin.

Kassan SS, Thomas TL, Moutsopoulos HM, Hoover R, Kimberly RP, Budman DR, Costa J, Decker JL and Chused TM (1978): Increased risk of lymphoma in Sjögren's syndrome. Ann Intern Med 89: 888–892.

Katz J, Marmary Y, Livneh A and Danon Y (1991): Drug allergy in Sjögren's syndrome (letter). Lancet 337: 239.

Kauppi M, Pukkala E and Isomäki H (1997): Elevated incidence of hematologic malignancies in patients with Sjögren's syndrome compared with patients with rheumatoid arthritis (Finland). Cancer Causes Control 8: 201–204.

Kelly CA, Foster H, Pal B, Gardiner P, Malcolm AJ, Charles P, Blair GS, Howe J, Dick WC and Griffiths ID (1991): Primary Sjögren's syndrome in north east England – a longitudinal study. Br J Rheumatol 30: 437–442.

Kilpi A, Bergroth V, Konttinen YT, Maury CPJ, Reitamo S and Wegelius O (1983): Lymphocyte infiltrations of the gastric mucosa in Sjögren's syndrome. Arthritis Rheum 26: 1196–2000.

Kishimoto T (1992): Interleukin-6 and its receptor in autoimmunity. J Autoimmun 5 Suppl A: 123–132.

Koivula T, Grönroos P, Gävert J, Icén A, Irjala K, Penttilä I, Siitonen A and Siukola OA (1990): Basic urinalysis and urine culture: Finnish recommendations from the working group on clean midstream specimens. Scand J Clin Lab Invest 50 Suppl 200: 26–33.

Konttinen YT, Kinnunen E, von Bonsdorff M, Lillqvist P, Immonen I, Bergroth V, Segerberg-Konttinen M and Friman C (1987): Acute transverse myelopathy successfully treated with plasmapheresis and prednisone in a patient with primary Sjögren's syndrome. Arthritis Rheum 30: 339–344.

Kriegsbaum N, von Linstow M, Oxholm P and Prause JU (1988): Keratoconjunctivitis sicca in patients with primary Sjögren's syndrome. A longitudinal study of ocular parameters. Acta Ophthalmol 66: 481–484.

Kruize AA, Hené RJ, van der Heide A, Bodeutsch C, de Wilde PCM, van Bijsterveld OP , deJong J, Feltkamp TEW, Kater L and Bijlsma JWJ (1996): Long-term follow-up of patients with Sjögren's syndrome. Arthritis Rheum 39: 297–303.

Lahdensuo A and Korpela M (1995): Pulmonary findings in patients with primary Sjögren's syndrome. Chest 108: 316–319.
Leroy JP, Pennec YL, Soulier C, Berthelot JM, Letoux G and Youinou P (1992): Follow up study of labial salivary gland lesions in primary Sjögren's syndrome. Ann Rheum Dis 51: 777–780.
Llorente L, Richaud-Patin Y, Fior R, Alcocer-Varela J, Wijdenes J, Fourrier BM, Galanaud P and Emilie D (1994): In vivo production of interleukin-10 by non-T cells in rheumatoid arthritis, Sjögren's syndrome, and systemic lupus erythematosus. A potential mechanism of B lymphocyte hyperactivity and autoimmunity. Arthritis Rheum 37: 1647–1655.
Manthorpe R, Oxholm P, Prause JU and Schiodt M (1986): The Copenhagen criteria for Sjögren's syndrome. Scand J Rheumatol Suppl 61: 19–21.
Markusse HM, Oudkerk M, Vroom TM and Breedveld FC (1992): Primary Sjögren's syndrome: clinical spectrum and mode of presentation based on an analysis of 50 patients selected from a department of rheumatology. Neth J Med 40: 125–134.
Martens PB, Pillemer SR, Jacobsson LT, O'Fallon WM and Matteson EI (1999): Survivorship in a population based cohort of patients with Sjögren's syndrome. J Rheumatol 26: 1296–1300.
Martin-Santos J, Carretero L, Armentia A, Alonso E and Gil I (1990): Hodgkin's disease occurring in primary Sjögren's syndrome. Ann Rheum Dis 49: 646–647.
Martinez-Lavin M, Vaughan JH and Tan EM (1979): Autoantibodies and the spectrum of Sjögren's syndrome. Ann Intern Med 91: 185–190.
Maury CPJ, Törnroth T and Teppo A-M (1985): Atrophic gastritis in Sjögren's syndrome. Arthritis Rheum 28: 388–394.
McCurdy D, Cornwell G and De Pratti V (1967): Hyperglobulinemic renal tubular acidosis: report of two cases. Ann Intern Med 67: 110–117.
McCurley T, Collins R, Ball E and Collins R (1990): Nodal and extranodal lymphoproliferative disorders in Sjögren's syndrome: a clinical and immunopathologic Study. Hum Pathol 21: 482–492.
Mellgren SI, Conn DL, Stevens JC and Dyck PJ (1989): Peripheral neuropathy in primary Sjögren's syndrome. Neurology 39: 390–394.
Meltzer M, Franklin EC, Ellias K, McCluskey RT and Cooper N (1966): Cryoglobulinemia. A clinical and laboratory study. II. Cryoglobulins with rheumatoid factor activity. Am J Med 40: 837–856.
Michalski J, Daniels T, Talal N and Grey H (1975): Beta-2 microglobulin and lymphocytic infiltration in Sjögren's syndrome. N Engl J Med 293: 1228–1231.
Miller SA, Dykes DD and Polesky HF (1988): A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16: 1215.
Morel P, Quiquandon I, Janin A, Dupriez B, Desablens B, Gosselin B and Bauters F (1993): High incidence of lymphoid infiltration on labial salivary gland biopsy in non-Hodgkin's lymphomas: clinical implications. Br J Haematol 85: 93–98.
Morris R and Fudenberg H (1967): Impaired renal acidification in patients with hypergamma-globulinemia. Medicine (Baltimore) 46: 57–69.
Moutsopoulos HM and Zerva LV (1990): Anti-Ro (SSA)/La (SSB) antibodies and Sjögren's syndrome. Clin Rheumatol 9 Suppl 1: 123–130.
Moutsopoulos H, Balow J, Cawley T, Stahl N, Autonovych T and Chused T (1978): Immune complex glomerulonephritis in sicca syndrome. Am J Med 64: 955–960.
Moutsopoulos HM, Webber BL, Vlagopoulos TP, Chused TM and Decker JL (1979): Differences in the clinical manifestations of sicca syndrome in the presence and absence of rheumatoid arthritis. Am J Med 66: 733–736.
Moutsopoulos HM, Steinberg AD, Fauci AS, Lane HC and Papadopoulos NM (1983): High incidence of free monoclonal lambda light chains in the sera of patients with Sjögren's syndrome. J Immunol 13: 2663–2665.
Moutsopoulos HM, Costello R, Drosos AA, Mavridis AK and Papadopoulos NM (1985): Demonstration and identification of monoclonal proteins in the urine of patients with Sjögren's syndrome. Ann Rheum Dis 44: 109–112.
Moutsopoulos HM, Cledes J, Skopouli FN, Elisaf M and Youinou P (1991): Nephrocalcinosis in Sjögren's syndrome: a late sequela of renal tubular acidosis. J Intern Med 230: 187–191.
Murube-del-Castillo J and Cortes-Rodrigo MD (1989): Eye parameters for the diagnosis of xerophthalmos. Clin Exp Rheumatol 7: 145–150.
Niemelä RK, Pikkujämsä SM, Hakala M, Huikuri HV and Airaksinen KEJ (2000): No signs of autonomic nervous system dysfunction in primary SS evaluated by 24 hour heart rate variability. J Rheumatol 27: 2605–10.

Norman ME, Feldman NI, Cohn RM, Roth KS and McCurdy DK (1978): Urinary citrate excretion in the diagnosis of distal renal tubular acidosis. J Pediatr 92: 394–400.
Osterberg T, Landahl DS and Hedegard M (1984): Salivary flow, saliva, pH and buffering capacity in 70 year old men and women. J Oral Rehabil 11: 157–170.
Ota T, Wake A, Eto S and Kobayashi T (1995): Sjögren's syndrome terminating in multiple myeloma. Scand J Rheumatol 24: 316–318.
Paganelli R, Quinti I, Fagiolo U, Cossarizza A, Ortolani C, Guerra E, Sansoni P, Pucillo LP, Scala E, Cozzi E, Bertollo L, Monti D and Franceschi C (1992). Changes in circulating B cells and immunoglobulin classes and subclasses in a healthy aged population. Clin Exp Immunol 90: 351–354.
Pasternack A and Linder E (1970): Renal tubular acidosis. An immunopathological study on four patients. Clin Exp Immunol 7: 115–123.
Pasternack A, Martio J, Nissilä M and Wegelius O (1970): Renal acidification and hypergamma-globulinaemia. A study of rheumatoid arthritis. Acta Med Scand 187: 123–127.
Pavlidis A, Karsh J and Moutsopoulos H (1982): The clinical picture of primary Sjögren's syndrome: a retrospective study. J Rheumatol 9: 685–690.
Pavlidis NA, Drosos AA, Papadimitriou C, Talal N and Moutsopoulos H (1992): Lymphoma in Sjögren's syndrome. Med Pediatr Oncol 20: 279–283.
Pease CT, Charles PJ, Shattles W, Markwick J and Maini RN (1993): Serological and immunogenetic markers of extraglandular primary Sjögren's syndrome. Br J Rheumatol 32: 574–577.
Pettersson T, Metsärinne K, Teppo AM and Fyhrquist F (1992): Immunoreactive interleukin-6 in serum of patients with B-lymphoproliferative diseases. J Intern Med 232: 439–442.
Pokorny G, Sonkodi S, Iványi B, Mohácsi G, Csáti S, Iványi T and Ormos J (1989): Renal involvement in patients with primary Sjögren's syndrome. Scand J Rheumatol 18: 231–234.
Pokorny G, Karácsony G, Lonovics J, Hudák J, Németh J and Varró V (1991): Types of atrophic gastritis in patients with primary Sjögren's syndrome. Ann Rheum Dis 50: 97–100.
Ponge T, Champetier de Ribes F, Ponge A, Garand R and Cottin S (1988): Chronic myelomonocytic leukemia and primary Sjögren's syndrome. Clin Rheumatol 7: 110–113.
Poux JM, Peyronnet P, Le Meur Y, Favereau JP, Charmes JP and Leroux-Robert C (1992): Hypokalemic quadriplegia and respiratory arrest revealing primary Sjögren's syndrome. Clin Nephrol 37: 189–191.
Price EJ and Venables PJ (1995): The etiopathogenesis of Sjögren's syndrome. Semin Arthritis Rheum 25: 117–133.
Przemioslo RT, Kontakou M, Nobili V and Ciclitira PJ (1994): Raised pro-inflammatory cytokines interleukin 6 and tumour necrosis factor alpha in coeliac disease mucosa detected by immunohistochemistry. Gut 35: 1398–1403.
Rajczy K, Vargha P and Beregi E (1986): Relationship between immunoglobulin levels and specific antibody titers in the elderly. Z Gerontol 19: 158–161.
Ramirez-Mata M, Pena-Ancira FF and Alarcon-Segovia D (1976): Abormal esophageal motility in primary Sjögren's syndrome. J Rheumatol 3: 63–69.
Ramos-Casals M, Cervera R, Font J, Garcia-Carrasco M, Espinosa G, Reino S, Pallares L and Ingelmo M (1998): Young onset of primary Sjögren's syndrome: clinical and immunological characteristics. Lupus 7: 202–206.
Raskin RJ, Tesar JT and Lawless OJ (1981): Hypokalemic periodic paralysis in Sjögren's syndrome. Arch Intern Med 141: 1671–1673.
Rhodus NL, Bloomquist C, Liljemark W and Bereuter J (1997): Prevalence, density and manifestations of oral Candida albicans in patients with Sjögren's syndrome. J Otolaryngol 26: 300–305.
Rice DH (1999): Chronic inflammatory disorders of the salivary glands. Otolaryngol Clin North Am 32: 813–818.
Riechmann P, D'Alessandro F, Nordan RP, Fauci AS and Kehrl JH (1991): IL-6 and tumor necrosis factor-alpha. Autocrine and paracrine cytokines involved in B cell function. J Immunol 146: 3462–3468.
Riesen W, Keller H, Skvaril F, Morell A and Barandun S (1976): Restriction of immunoglobulin heterogeneity, autoimmunity and serum protein levels in aged people. Clin Exp Immunol 26: 280–285.
Rustin MHA, Isenberg DA, Griffiths MH and Gilkes JJH (1988): Sjögren's syndrome and pleomorphic T-cell lymphoma presenting with skin involvement. J R Soc Med 81: 47–49.
Schein OD, Hochberg MC, Munoz B, Tielsch JM, Bandeen-Roche K, Provost T, Anhalt GJ and West S (1999): Dry eye and dry mouth in the elderly: a population-based assessment. Arch Intern Med 159: 1359–1363.

Schuurman HJ, Gooszen HC, Tan IWN, Kluin PM, Wagenaar SS and van Unnik JAM (1987): Low-grade lymphoma of immature T-cell phenotype in a case of lymphocytic interstitial pneumonia and Sjögren's syndome. Histopathology 11: 1193–1204.

Shahar I, Fireman E, Topilsky M, Grief J, Kivity S, Spirer Z and Ben Efraim S (1996): Effect of IL-6 on alveolar fibroblast proliferation in interstitial lung diseases. Clin Immunol Immunopathol 79: 244–251.

Shearn MA (1971): Sjögren's syndrome. In: Major problems in internal medicine, vol. II. WB Saunders, Philadelphia.

Shearn MA (1977): Sjögren's syndrome. Med Clin North Am 61: 271–282.

Shearn M and Tu W (1965): Nephrogenic diabetes insipidus and other defects of renal tubular function in Sjögren's syndrome. Am J Med 39: 312–318.

Shearn MA and Tu WH (1968): Latent renal tubular acidosis in Sjögren's syndrome. Ann Rheum Dis 27: 27–32.

Shin S, Sheibani K, Fishleder A, Ben-Ezra J, Bailey A, Koo CH, Burke JS, Tubbs R and Rappaport H (1991): Monocytoid B-cell lymphoma in patients with Sjögren's syndrome: a clinicopathologic study of 13 patients. Hum Pathol 22: 422–430.

Shioji R, Furuyama T, Onodera S, Saito H, Ito H and Sasaki Y (1970): Sjögren's syndrome and renal tubular acidosis. Am J Med 48: 456–463.

Shiozawa S, Shiozawa K, Shimizu S, Nakada M, Isobe T and Fujita T (1987): Clinical studies of renal disease in Sjögren's syndrome. Ann Rheum Dis 46: 768–772.

Siamopoulos K, Mavridis A, Elisaf M, Drosos A and Moutsopoulos H (1986): Kidney involvement in primary Sjögren's syndrome. Scand J Rheumatol Suppl 61: 156–160.

Siamopoulos K, Elisaf M, Drosos A, Mavridis A and Moutsopoulos H (1992): Renal tubular acidosis in primary Sjögren's syndrome. Clin Rheumatol 11: 226–230.

Siamopoulos KC, Elisaf M and Moutsopoulos HM (1994): Hypokalaemic paralysis as the presenting manifestation of primary Sjögren's syndrome. Nephrol Dial Transplant 9: 1176–1178.

Simmons-O'Brien E, Chen S, Watson R, Antoni C, Petri M, Hochberg M, Stevens MB and Provost TT (1995): One hundred anti-Ro (SS-A) antibody positive patients: a 10 year follow-up. Medicine (Baltimore) 74: 109–130.

Sjögren H (1933): Zur Kenntniss von Keratoconjunctivitis sicca (Keratitis filiformis bei Hypofunktion der Tränendrüsen). Acta Ophthalmol Suppl 11: 1–151.

Skopouli FN, Drosos AA, Papaioannu T and Moutsopoulos HM (1986): Preliminary diagnostic criteria for Sjögren's syndrome. Scand J Rheumatol Suppl 61: 22–25.

Skopouli FN, Talal A, Galanopoulou V, Tsampoulas CG, Drosos AA and Moutsopoulos HM (1990): Raynaud's phenomenon in primary Sjögren's syndrome. J Rheumatol 17: 618–620.

Skopouli FN, Dafni U, Ioannidis JP and Moutsopoulos HM (2000): Clinical evolution, and morbidity and mortality of primary Sjögren's syndrome. Semin Arthritis Rheum 29: 296–304.

Sobel JD, Taylor Z, Alroy G, Lichtig C and Valero A (1977): Systemic lupus erythematosus, Sjögren's syndrome and glomerular nephritis. Postgrad Med J 53: 97–101.

Strimlan CV, Rosenov EC, Divertie MB and Harrison EG (1976): Pulmonary manifestations of Sjögren's syndrome. Chest 70: 354–361.

Ström T, Evrin P-E and Karlsson A (1978): Serum beta-2-microglobulin in Sjögren's syndrome. Scand J Rheumatol 7: 97–100.

Sutcliffe N, Inanc M, Speight P and Isenberg D (1998): Predictors of lymphoma development in primary Sjögren's syndrome. Semin Arthritis Rheum 28: 80–87.

Talal N (1971): Sjögren's syndrome, lymphoproliferation and renal tubular acidosis. Ann Intern Med 74: 633–634.

Talal N (1993): Sjögren's syndrome and connective tissue diseases associated with other immunologic disorders. In: Arthritis and allied conditions. A textbook of rheumatology, 12th ed., pp. 1343–1356. Eds. DJ McCarty and WJ Koopmann, Lea and Febiger, Philadelphia.

Talal N and Bunim JJ (1964): Development of malignant lymphoma in the course of Sjögren's syndrome. Am J Med 36: 529–540.

Talal N, Sokoloff L and Barth WF (1967): Extrasalivary lymphoid abnormalities in Sjögren's syndrome (reticulum cell sarcoma, "pseudolymphoma", and macroglobulinemia). Am J Med 43: 50–65.

Talal N, Zisman E and Schur P (1968): Renal tubular acidosis, glomerulonephritis and immunologic factors in Sjögren's syndrome. Arthritis Rheum 11: 774–786.

Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N and Winchester RJ (1982): The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 118: 412–416.

Tannen RL (1971): The response of normal subjects to the short ammonium chloride test: the modifying influence of renal ammonia production. Clin Sci 41: 583–595.

Tatsumi H, Tateno S, Hiki Y, Shigematsu H and Kobayashi Y (1998): Crescentic glomerulonephritis associated with membranous nephropathy in a case with primary Sjögren's syndrome. Nephrol Dial Transplant 13: 2623–2627.

Thomas E, Hay EM, Hajeer A and Silman AJ (1998): Sjögren's syndrome: a community-based study of prevalence and impact. Br J Rheumatol 37: 1069–1076.

Tishler M, Paran D and Yaron M (1998a): Allergic disorders in primary Sjögren's syndrome. Scand J Rheumatol 27: 166–169.

Tishler M, Yaron I, Geyer O, Shirazi I, Naftaliev E and Yaron M (1998b): Elevated tear interleukin-6 levels in patients with Sjogren syndrome. Ophthalmology 105: 2327–2329.

Tishler M, Yaron I, Shirazi I, Yossipov Y and Yaron M (1999): Increased salivary interleukin-6 levels in patients with primary Sjögren's syndrome. Rheumatol Int 18: 125–127.

Tu W, Shearn M, Lee J and Hopper J (1968): Interstitial nephritis in Sjögren's syndrome. Ann Intern Med 69: 1163–1170.

Tzioufas A and Moutsopoulos H (1998): Sjögren's syndrome. In: Rheumatology, 2nd ed., pp. 7.32.1–7.32.12. Eds. S Klippel and P Dieppe, Mosby International, London.

Tzioufas AG, Manoussakis MN, Costello R, Silis M, Papadopoulos NM and Moutsopoulos H (1986): Cryoglobulinemia in autoimmune rheumatic diseases: Evidence of circulating monoclonal cryoglobulins in patients with primary Sjögren's syndrome. Arthritis Rheum 29: 1098–1104.

Tzioufas AG, Moutsopoulos HM and Talal N (1987): Lymphoid malignancy and monoclonal proteins. In: Sjögren's syndrome. Clinical and immunological aspects, pp. 129–136. Eds. N Talal, HM Moutsopoulos and SS Kassan, Springer-Verlag, Berlin.

Tzioufas A, Boumba D, Skopouli F and Moutsopoulos H (1996): Mixed monoclonal cryoglobulinemia and monoclonal rheumatoid factor cross-reactive idiotypes as predictive factors for the development of lymphoma in primary Sjögren's syndrome. Arthritis Rheum 39: 767–772.

Ulirsch RC and Jaffe ES (1987): Sjögren's syndrome-like illness associated with the acquired immunodeficiency syndrome related complex. Hum Pathol 18: 1063–1068.

Van der Valk PGM, Hollema H, van Voorst Vander PC, Brinker M and Poppema S (1989): Sjögren's syndrome with specific cutaneous manifestations and multifocal clonal T-cell populations progressing to a cutaneous pleomorphic T-cell lymphoma. Am J Clin Pathol 92: 357–361.

Venables PJW, Shattles W, Pease CT, Ellis JE, Charles PJ and Maini RN (1989): Anti-La (SS-B): a diagnostic criterion for Sjögren's syndrome? Clin Exp Rheumatol 7: 181–184.

Viergever P and Swaak T (1991): Renal tubular dysfunction in primary Sjögren's syndrome: clinical studies in 27 patients. Clin Rheumatol 10: 23–27.

Vitali C and Bombardieri S (1997): The European Classification criteria for Sjögren's syndrome (SS): proposal for modification of the rules for classification suggested by the analysis of receiver operating characteristics (ROCS) curve of the criteria performance. J Rheumatol 24 Suppl: S18.

Vitali C, Tavoni A , Sciuto M, Macceroni M, Moriconi L and Bombardieri S (1991): Renal involvement in primary Sjögren's syndrome: a retrospective-prospective study. Scand J Rheumatol 20: 132–136.

Vitali C, Bombardieri S, Moutsopoulos HM, Balestrieri G, Bencivelli W, Bernstein RM, Bjerrum KB, Braga S, Coll J, de Vita S, Drosos AA, Ehrenveld M, Hatron PY, Hay EM, Isenberg DA, Janin A, Kalden JR, Kater L, Konttinen YT, Maddison PJ, Maini RN, Manthorpe R, Meyer O, Ostuni P, Pennec Y, Prause JU, Richards A, Sauvezie B, Schiodt M, Sciuto M, Scully C, Shoenfeld Y, Skopouli FN, Smolen JS, Snaith ML, Tishler M, Todesco S, Valesini G, Venables PJW, Wattiaux MJ and Youinou P (1993): Preliminary criteria for the classification of Sjögren's syndrome: results of a prospective concerted action supported by the European Community. Arthritis Rheum 36: 340–347

Vivancos J, Bosch X, Grau J, Coca A and Font J (1992): Development of Hodgkin's disease in the course of primary Sjögren's syndrome. Br J Rheumatol 31: 561–563.

Voulgarelis M, Dafni UG, Isenberg DA and Moutsopoulos HM (1999): Malignant lymphoma in primary Sjögren's syndrome: a multicenter, retrospective, clinical study by the European concerted action on Sjögren's syndrome. Arthritis Rheum 42: 1765–1772.

Wang SL, Zou ZJ, Yu SF and Zhu JR (1993): Recurrent swelling of parotid glands and Sjögren¨s syndrome. Int J Oral Maxillofac Surg 22: 362–365.

Weise M, Prüfer D, Jaques G, Keller M and Mondorf AW (1981): β_2-microglobulin and other proteins as parameter for tubular function. Contrib Nephrol 24: 88–98.
Whaley K, Goudie RB, Williamson J, Nuki G, Dick WC and Buchanan WW (1970): Liver disease in Sjögren's syndrome and rheumatoid arthritis. Lancet 1: 861–863.
Whaley K, Webb J, McAvoy BA, Hughes GR, Lee P, MacSween RN and Buchanan WW (1973a): Sjögren's syndrome 2. Clinical associations and immunological phenomena. QJM 42: 513–548.
Whaley K, Williamson J, Chisholm DM, Webb J, Mason DK and Buchanan WW (1973b): Sjögren's syndrome 1. Sicca components. QJM 42: 279–304.
Wibell L, Evrin P-E and Berggård I (1973): Serum β_2-microglobulin in renal disease. Nephron 10: 320–331.
Wilke WS, Tubbs RR, Bukowski RM, Currie TE, Calabrese LH, Weiss RA, Savage RA and Sebek BA (1984): T cell lymphoma occurring in Sjögren's syndrome. Arthritis Rheum 27: 951–955.
Winer R (1993): Sjögren's syndrome. In: The kidney in collagen vascular diseases, pp. 179–187. Eds. E Grisham, J Churg, MA Needle and VS Venkataseshan, Raven Press Ltd, New York.
Winer RL, Cohen AH, Sawhney AS and Gorman JT (1977): Sjögren's syndrome with immune-complex tubulointerstitial renal disease. Clin Immunol Immunopathol 8: 494–503.
Wrong O and Davies HEF (1959): The excretion of acid in renal disease. QJM 28: 259–313.
Youinou P, Pennec Y-L, Katsikis P, Jouquan J, Fauquert P and Le Goff P (1990): Raynaud's phenomenon in primary Sjögren's syndrome. Br J Rheumatol 29: 205–207.
Yu H, Yanagisawa Y, Forbes M, Cooper E, Crockson R and MacLennan I (1983): Alpha-1-microglobulin: an indicator protein for renal tubular function. J Clin Pathol 36: 253–259.
Zufferey P, Meyer OC, Grossin M and Kahn MF (1995): Primary Sjögren's syndrome (SS) and malignant lymphoma. Scand J Rheumatol 24: 342–345.

Original publications

The permission of the following publishers to reprint the original publications is acknowledged:

BMJ Publishing Group, London, United Kingdom (I, II)

Oxford University Press, Oxford, United Kingdom (III, V)

Dustri-Verlag, Dr.Karl Feistle, Deisenhofen, Germany (IV)