Osteoporosis in SLE

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Abstract

As the treatment of Systemic Lupus Erythematosus (SLE) has become more effective the focus has partly shifted from main concern of short term morbidity and survival to long term complications such as osteoporosis. The aims of this thesis were to a) determine prevalence and risk factors of osteoporosis and of b) vertebral fractures c) evaluate if adequate osteoporotic treatment was provided d) determine if resistin, an adipokine with proposed pro-inflammatory properties, was associated with markers of inflammation or bone mineral density (BMD) and to e) investigate patients self reported health related quality of life (HRQOL) and its relationship to disease variables and employment status in female SLE patients.

In this cross sectional study 163 female patients with SLE were examined during the winter and spring 2002-2003.

BMD was significantly reduced in patients compared to expected calculated reference values. Bisphosphonates were taken by 35% of patients with osteoporosis and 36% of patients with osteoporosis and/or osteopenia and concomitant glucocorticosteroid medication. Factors associated with low BMD in SLE were markers of inflammation, impaired kidney function and disease damage in addition to the conventional risk factors, high age and low weight. Glucocorticosteroid, current and cumulative doses, were associated with BMD in simple but not in multiple regression models.
Only 6 (4%) women had a history of a clinical vertebral fracture whereas 29% had radiological fractures. High age was the strongest risk factor of vertebral fracture. There were no significant differences regarding SLE specific variables or current or cumulative glucocorticosteroid doses between patients with or without vertebral fractures.

The SLE patients scored their HRQOL significantly lower than age and sex matched references in all SF-36 subscales. Prevalent vertebral fractures did not have a major impact on HRQOL. In patients 64 years old or younger (n=142) 54% worked full or part time. Working ability was associated with low age and high scores (indicating better health) in physical SF-36 subscales.

Serum levels of resistin did not differ between patients and controls. There were clear associations between high resistin levels and general inflammation, renal disease,

treatment with glucocorticosteroids and bone loss in the SLE patient group. Resistin was independently associated to inflammation in multiple logistic regression analyses.

In conclusion, our results show that female patients with SLE have increased risk of low BMD and osteoporosis and few patients are treated adequately. Vertebral fractures are common but seldom diagnosed. More attention should also be given factors of importance to the patients HRQOL, which is scored considerably lower than in general population. We suggest that resistin has pro-inflammatory properties in SLE and possibly also influence bone quality negatively.

Keywords: Systemic lupus erythematosus, bone mineral density, osteoporosis, vertebral fracture, health-related quality of life, SF-36, resistin, cross sectional study

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List of publications

This thesis is based on the following papers, which are referred to in the text by their Roman numerals (I-IV):

I. K. Almehed, H. Forsblad d'Elia, G. Kvist, C. Ohlsson, H. Carlsten
Prevalence and risk factors of osteoporosis in female SLE patients--extended report
Rheumatology 2007;46;1185–1190

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Prevalence and risk factors of vertebral compression fractures in female SLE patients.
Submitted

III. K. Almehed , H. Forsblad d'Elia, H. Carlsten
Health related quality of life in Systemic Lupus Erythematosus and its association to disease and work disability.
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IV. K. Almehed, H. Forsblad d'Elia, M. Bokarewa and H. Carlsten
Role of resistin as a marker of inflammation in Systemic Lupus Erythematosus.
Arthritis Research & Therapy 2008;10(1):R15

Contents

Abbreviations

BILAG	British Isles Lupus Activity Group
BMD	Bone mineral density
BMP	Bone morphogenetic proteins
BMU	bone remodelling unit
CNS	Central nervous system
DKK-1	Dickkopf-1
EBV	Epstein Barr Virus
ECLAM	European Consensus Lupus Activity Measure
ELISA	Enzyme-linked immunosorbent assay
HRQOL	Health related quality of life
HRT	Hormone replacement therapy
ICTP	C-terminal telopeptide of type I collagen
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
M-CSF	Macrophage colony-stimulating factor
OCP	Osteoclast progenitor
OPG	Osteoprotegrin
PICP	C-terminal propeptide of type I procollagen
PINP	N-terminal propeptide of type I procollagen
PTH	Parathyroid hormone
QCT	Quantitative computed tomography
RA	Rheumatoid arthritis
RANK	Receptor activator of NFκB
RANKL	Receptor activator of NFκB ligand
RIA	Radioimmunoassay
SD	Standard deviation
SF-36	Medical Outcome Study Short Form-36
SLAM	Systemic Lupus Activity Measure
SLE	Systemic lupus erythematosus
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index
SLEDAI-2K	Systemic Lupus Erythematosus Disease Activity Index 2000
SLICC/ACR	Systemic Lupus International Collaborative Clinics/ American Collage of Rheumatology damage index
TGF	Transforming growth factor
Th	T helper
TNF	Tumor necrosis factor
WHO	World Health Organisation
Wnt	Wingless

Systemic Lupus Erythematosus

SLE is an intriguing disease often considered as a model-disease for autoimmunity. The common existence of auto-antibodies directed against double-stranded DNA, the keeper of our genetic information, most certainly contributes to the fascination of the disease. SLE flares give rise to several distinct and important disease manifestations. I will however address aspects primarily connected with long term disease, osteoporosis and vertebral fractures. I will also discuss factors, disease-related or demographic; those are associated to and may contribute to the evolvement of low BMD and vertebral fractures. Special interest is laid on resistin, an inflammation marker and pro-inflammatory cytokine. I will also focus on variables important to health related quality of life (HRQOL) in SLE.

Clinical aspects and outcome

SLE is a chronic autoimmune disease mainly affecting women. Female to male ratio 5-10:1 [1]. The annual incidence in a Southern Swedish population is approximately 4,5/100000 and the prevalence 68/100000 [2]. The SLE prevalence differs worldwide with the highest prevalence in black populations. This can reflect methodological differences but also be suggestive of the aetiology of the disease.

The disease is characterized by production of a variety of auto-antibodies and multi-organ systems involvement. Typical serological findings include anti-nuclear (ANA), anti-double stranded DNA (anti-DNA) and anti-Smith (anti-Sm) antibodies. These are also included in the disease criteria [3]. Several different auto-antibodies have been shown to play a role in clinical manifestations. Especially high affinity anti-bodies are thought to be of clinical relevance [4]. One tissue damaging mechanism in SLE is proposed to be caused by anti-DNA binding to DNA-containing debris in the blood stream. The DNA released from apoptotic cells in the form of nucleosomes form immune complexes together with anti-DNA. These settle in the glomerular basement membrane, activating the complement system which causes inflammation and tissue damage [5, 6]. Another disease mechanism includes IgG auto-antibodies directed to cell surface antigens situated, for example, on red blood cells or thrombocytes. Fc receptors on macrophages bind to and will clear these opsonised cells by phagocytosis resulting in haemolytic anaemia or thrombocytopenia [7, 8]. Yet other antibodies, anti-cardiolipin antibodies, affect the patient by interfering with the coagulation haemostasis increasing the risk of thrombosis and embolies [9].

A Canadian survey of SLE manifestations at anytime during disease course showed following manifestations and frequencies; arthralgia (85%), skin rash including sun sensitivity and the typical malar or facial butterfly-rash (78%), constitutional symptoms (77%), renal involvement (74%), arthritis (63%), Raynaud´s phenomenon

(60%), vasculitis (56%), central nervous system affection (54%), mucous membrane affection (52%), lymphadenopathy (32%), pleuritis (30%), pericarditis (23%) [10].

The disease course is varied. For some patients SLE flares are rare and mild, but an Italian study showed that 44% of the patients experienced at least one severe flare (defined as glomerulonephritis, major CNS or heart and lung manifestation, haemolytic or aplastic anaemia) during 15 years follow up [11]. Survival rates have improved during the last 50 years reflecting not only earlier diagnose in milder cases, but also the possibility of more intensive immunosuppressive treatment when needed. More, but not enough, attention is given to long-term disease morbidity like renal failure, hypertension and cardiovascular disease. Vigilance for infections and access to effective antibiotics is also important in SLE since the treatment, often including cytotoxic substances and glucocorticosteroids, increase the susceptibility to infections [12].

Survival rates 5, 10 and 15 years from the diagnosis, presented in an Italian study, show 96%, 93% and 76% survival respectively[11]. Mortality was higher in patients suffering from inner organ affection than those with mild flares exclusively. A Danish retrospective study presented in 1999, showed a 4,6-fold increased mortality compared with general population [13]. In a Swedish survey only mortality after 10 years disease duration exceeded that in an age and sex matched population. Atherosclerotic vascular disease, active disease manifestations and infections contributed to mortality [2]. These causes of mortality were also confirmed in multinational studies of current causes of death in SLE [14, 15].

Classification criteria

Because of the varying SLE manifestations the diagnosis is based on criteria. The first set of criteria were defined in 1971 by the American Rheumatism Association and later revised in 1982 [3]. At least 4 of 11 criteria must be present, but not necessarily at the same time, table 1. The criteria were developed mainly for the purpose of clinical studies. The sensitivity and specificity for the criteria are 96% respectively. In clinical praxis SLE diagnosis is considered in a patient with auto-antibodies and autoimmune disease manifestations typical for SLE from at least two different organ systems, in the absence of a more plausible diagnosis.

Table 1.

Criteria for classification of SLE. For identifying patients in clinical studies 4 or more of the 11 criteria must be present, serially or simultaneously.

Criterion	Definition
1. Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
2. Discoid rash	Erythematosus raised patches with adherent keratotic scaling and follicular plugging; atrophic scaring may occur
3. Photosensitivity	Skin rash as result of unusual reaction to sunlight by patient history or physician observation
4. Oral ulcers	Oral or nasopharyngeal, usually painless, observed by physician
5. Arthritis	Non-erosive arthritis involving two or more peripheral joints
6. Serositis	Pleuritis OR pericarditis
7. Renal disorder	Persistent proteinuria >0,5g/day or greater than 3+ if quantification not performed OR Cellular casts
8. Neurologic disorder	Seizures OR psychosis, in the absence of offending drugs or known metabolic derangements
9. Hematologic disorder	Haemolytic anaemia OR leukopenia (≥2 occasions) OR lymphopenia (≥2 occasions) OR thrombocytopenia (in the absence of offending drugs)
10. Immunologic disorder □	Anti-DNA (native DNA) OR anti-Sm OR anti-phospholipid antibodies
11. Antinuclear antibody	

□ Modifications were made in 1997 [16]

Aetiology and Pathogenesis

SLE is a multifactorial disease. Genetic, hormonal and environmental factors are predisposing or contributing to disease development. There are racial differences with two to four-fold higher prevalence in non-Caucasian compared to Caucasian population [1]. The concordance for SLE in monozygotic twins is 25% and about 2 % in dizygotic twins [17]. Several gene-loci with probable linkage to SLE are known [6]. Many of these genes share the property of coding for different components in the immune system. Genes involved in antigen presentation, inhibition of lymphocyte activation, apoptosis and clearance of immune complexes are examples of genes where variants

are associated with SLE [18]. It is also known that deficiencies of factors early in the complement system frequently is associated with SLE [19] .

Female sex-hormones, estrogens, are thought to contribute to the increasing incidence in SLE during female puberty and fertile ages [20 21]. It has been shown that estrogen-containing contraceptives [22] and hormone replacement therapy (HRT) [23] slightly increase the risk of later developing SLE. Estrogens can also affect existing SLE. Contraceptives have not been found to increase the rate of severe flares in mild SLE [24]. HRT has been reported to increase mild to moderate flares [25]. However patients with phospholipid antibody syndrome and active renal disease were not included in the two latter studies. Estradiol affect the immune system in several ways [26 27]. In murine SLE models, estradiol aggravates glomerulonephritis and can block the negative selection of naïve B-cells and thus enhance autoantibody production [28, 29].

Several environmental factors have been mentioned as possible trigger factors of disease or SLE flares. Sunlight-exposure of sun-reactive skin is thought to trigger flares through events starting with DNA damage and increased apoptosis [30]. Other environmental factors may influence individuals in favour of lupus, if specific genetic variants exist, or if applied at specific time points during life [31]. Factors with certain or possible influence before disease onset are frequent partake of alfalfa sprouts [32], smoking and getting blood transfusions [33], exposure to organic solvents, heavy metals, silica or aromatic amines [34]. Epstein Barr Virus (EBV) is one of several infections of possible importance to SLE development [35].

Assessment of disease activity and disease damage in SLE

SLE disease activity changes during time. To be able to measure and compare disease activity from time to time, for example when evaluating different medications or in clinical trials, different disease activity indices have been created. They transform disease manifestations and laboratory aberrations into numerals. There are several disease activity indices in routine clinical use. They have been validated and compared with each other [36]. The British Isles Lupus Activity Group (BILAG) is an extensive index comprising eight organ domains. The organ manifestations are assessed as either non existent, new, worse or better [37]. BILAG is often used in pharmaceutical testing. Another index is Systemic Lupus Activity Measure (SLAM) an index of medium length measuring changes in a broad spectrum of disease manifestations quantitatively over time [38 39]. European Concensus Lupus Activity Measure (ECLAM) [40] and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) are other instruments for SLE activity. SLEDAI-2K is a short instrument that measures ongoing, new or recurrent activity, as recognized by the clinician, during the last 10 days [41], table 2. This is a minor modification of the original SLEDAI where only new or recurrent manifestations were registered. There is a high correlation between the two versions of SLEDAI (r=0,97) [42]. All manifestations should be caused by SLE and

each manifestation gives a score. SLEDAI-2K was used as disease activity measurement in the patient group presented in this thesis because it is widely used and easy to handle by both experts and trainees [43]. It gives one index score per patient which is practical in further statistical analyses. The median (range) SLEDAI-2K value for the patients was 5(0-31) and mean value (SD) 6,7(6,0) (I-IV).

The assessment of SLE prognosis requires, in addition to disease activity measures, an estimation of the organ damage caused by SLE. Systemic Lupus International Collaborative Clinics/ American Collage of Rheumatology damage index (SLICC/ACR), is the only widely used damage index [44], table 3. In this assessment specified irreversible damage in 12 organs or systems, occurring after the SLE diagnose and having been present for at least 6 months, are recorded. SLICC/ACR is validated and reproducible between different observers [44] and therefore often used in clinical trials and clinical work. SLICC can however not distinguish between damage caused by SLE, its therapy or a concurrent disease but has shown to be associated with morbidity and mortality in several studies [45] [14].

Table 2. SLEDAI-2K. Enter weight in SLEDAI score column if descriptor is present at the time of the visit or in the preceding 10 days.

Weight	SLEDAI SCORE	Descriptor	Definition
8	□	Seizure	Recent onset. Exclude metabolic, infectious or drug cause
8	□	Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucinations, incoherence, marked loose associations, impoverished thought content, marked illogical thinking, bizarre, disorganized, or catatonic behaviour. Excluded uremic and drug causes.
8	□	Organic Brain Syndrome	Altered mental function with impaired orientation, memory or other intelligent function, with rapid onset fluctuating clinical features. Include clouding of consciousness with reduced capacity to focus, and inability to sustain attention to environment, plus at least two of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, or increased or decreased psychomotor activity. Exclude metabolic, infectious or drug causes.
8	□	Visual Disturbance	Retinal changes of SLE. Include cytoid bodies, retinal haemorrhages, serious exudates or haemorrhages in the choroids, or optic neuritis. Exclude hypertension, infection, or drug causes.
8	□	Cranial Nerve Disorder	New onset of sensory or motor neuropathy involving cranial nerves.
8	□	Lupus Headache	Severe persistent headache: may be migrainous, but must be nonresponsive to narcotic analgesia.
8	□	CVA	New onset of cerebrovascular accident(s). Exclude arteriosclerosis
8	□	Vasculitis	Ulceration, gangrene, tender finger nodules, periungual, infarction, splinter haemorrhages, or biopsy or angiogram proof of vasculitis
4	□	Arthritis	More than 2 joints with pain and signs of inflammation (i.e. tenderness, swelling, or effusion).
4	□	Myositis	Proximal muscle aching/weakness, associated with elevated creatine phosphokinase/adolase or electromyogram changes or a biopsy showing myositis.
4	□	Urinary Casts	Haeme-granular or red blood cell casts
4	□	Hematuria	>5 red blood cells/high power field. Exclude stone, infection or other cause.
4	□	Proteinuria	>0.5 g/24 hours.
4	□	Pyuria	>5 white blood cells/high power field. Exclude infection.
2	□	Rash	Inflammatory type rash.
2	□	Alopecia	Abnormal, patchy or diffuse loss of hair.
2	□	Mucosal Ulcers	Oral or nasal ulcerations
2	□	Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening.
2	□	Pericarditis	Pericardial pain with at least 1 of the following: rub, effusion, or electrocardiogram confirmation.
2	□	Low Complement	Decrease in CH50, C3, or C4 below the lower limit of normal for testing laboratory.
2	□	Increased DNA binding	Increased DNA binding by Farr assay above normal range for testing laboratory.
1	□	Fever	>38°C. Exclude infectious cause
1	□	Thrombocytopenia	<100,000 platelets/mm3, exclude drug cause.
1	□	Leukopenia	<3,000 White blood cell/mm3, exclude drug causes.

Table 3. SLICC/ACR.

Item	Score
Ocular (either eye, by clinical assessment)	
Any cataract ever	1
Retinal change *or* optic atrophy	1
Neuropsychiatric	
Cognitive impairment (e.g., memory deficit, difficulty with calculation, poor concentration, difficulty in spoken or written language, impaired performance level) *or* major psychosis	1
Seizures requiring therapy for 6 months	1
Cerebrovascular accident ever (score 2 if >1)	1 (2)
Cranial or peripheral neuropathy (excluding optic)	1
Transverse myelitis	1
Renal	
Estimated or measured glomerular filtration rate <50%	1
Proteinuria ≥3.5 gm/24 hours	1
or	
End-stage renal disease (regardless of dialysis or transplantation)	3
Pulmonary	
Pulmonary hypertension (right ventricular prominence, or loud P2)	1
Pulmonary fibrosis (physical and radiograph)	1
Shrinking lung (radiograph)	1
Pleural fibrosis (radiograph)	1
Pulmonary infarction (radiograph)	1
Cardiovascular	
Angina *or* coronary artery bypass	1
Myocardial infarction ever (score 2 if >1)	1 (2)
Cardiomyopathy (ventricular dysfunction)	1
Valvular disease (diastolic, murmur, or systolic murmur >3/6)	1
Pericarditis for 6 months, *or* pericardiectomy	1
Peripheral vascular	
Claudication for 6 months	1
Minor tissue loss (pulp space)	1
Significant tissue loss ever (e.g., loss of digit or limb) (score 2 if >1 site)	1 (2)
Venous thrombosis with swelling, ulceration, *or* venous stasis	1
Gastrointestinal	
Infarction or resection of bowel below duodenum, spleen, liver, or gall bladder ever, for cause any (score 2 if >1 site)	1 (2)
Mesenteric insufficiency	1
Chronic peritonitis	1
Stricture *or* upper gastrointestinal tract surgery ever	1
Musculoskeletal	
Muscle atrophy or weakness	1
Deforming or erosive arthritis (including reducible deformities, excluding avascular necrosis)	1
Osteoporosis with fracture or vertebral collapse (excluding avascular necrosis)	1
Avascular necrosis (score 2 if >1)	1 (2)
Osteomyelitis	1
Skin	
Scarring chronic alopecia	1
Extensive scarring or panniculum other than scalp and pulp space	1
Skin ulceration (excluding thrombosis) for >6 months	1
Premature gonadal failure	1
Diabetes (regardless of treatment)	1
Malignancy (exclude dysplasia) (score 2 if >1 site)	1 (2)

* Damage (nonreversible change, not related to active inflammation) occurring since onset of lupus, ascertained by clinical assessment and present for at least **6 months** unless otherwise stated. Repeat episodes must occur at least 6 months apart to score 2. The same lesion cannot be scored twice.

Bone biology

Bone structure and function

The skeleton is vital for body posture, mobility and protection of inner organs. The maximum bone mass is reached at 25-30 years of age. There is a constant bone turnover and approximately 10% of the bone mass is exchanged every year. Bone resorption and formation exist simultaneously. The state of equilibrium can be dislocated for example in higher ages, by use of medication or bad nourishment, resulting in net loss of bone mass. There are two types of bone, the dense outer lining consisting of cortical bone and the porous, honeycombed appearing inner trabecular bone. Together they form a fairly light but hard and strong entity. The proportions of cortical and trabecular bone varies in different parts of the skeleton which affects the biomechanical characteristics and the turn over rate since trabecular bone is more metabolic active then cortical bone. Bone contains four cell types and an extra cellular matrix. The cells are osteoblasts, osteocytes, bone lining cells and osteoclasts. Ninety % of the organic bone matrix consists of collagen type I. Type I collagen can also be found in lower amounts in blood vessels, cornea, dentin, skin and tendon. In bone the type I collagen precursor is secreted by osteoblasts and forms a triple helix with extended carboxyterminal and aminoterminal ends. These are cleaved during secretion and a collagen fibril is formed. The collagen fibrils are subsequently mineralised by calcium-hydroxyapatit and other calcium salts. Type I collagen contribute to the elasticity of the bone which otherwise would be hard and brittle.

Bone cells

The osteoclasts resorb bone. Osteoclasts are derived from a hematopoetic cell linage, like macrophages, monocytes and dendritic cells. Under the influence of macrophage stimulating factor (M-CSF) and receptor activator of NFκB ligand (RANKL) the activated multi nuclear osteoclast is formed from a progenitor. Osteoblasts are responsible for bone formation and regulation of osteoclast differentiation. They are derived from mesenchymal stem cells which also are the progenitors of adipocytes, chondrocytes, myocytes and fibroblasts in the bone marrow stroma. Some of the factors of importance for osteoblast differentiation are bone morphogenetic proteins (BMP), transforming growth factor (TGFβ) and wingless (Wnt) proteins responsible also for aspects of osteoblast cell growth and function [46 47]. Mature osteoblasts restore bone by forming the osteoid. Osteoid contains bone matrix proteins like collagen type I and bone morphogenetic proteins (BMP). Osteoblasts also initiate the mineralisation or calcification of the osteoid by membrane bound alkaline phosphatase. In the process of mineralisation some osteoblasts are trapped in the new bone and become osteocytes. The osteocytes are sensitive to mechanical loading and initiate and signal need for bone remodelling in response to the loading and when micro-fractures occur [48]. Bone-lining cells are derived from osteoblasts and lie on mineralised bone surface

maintaining the microenvironment and possibly also initiating remodelling when needed. Figure 1.

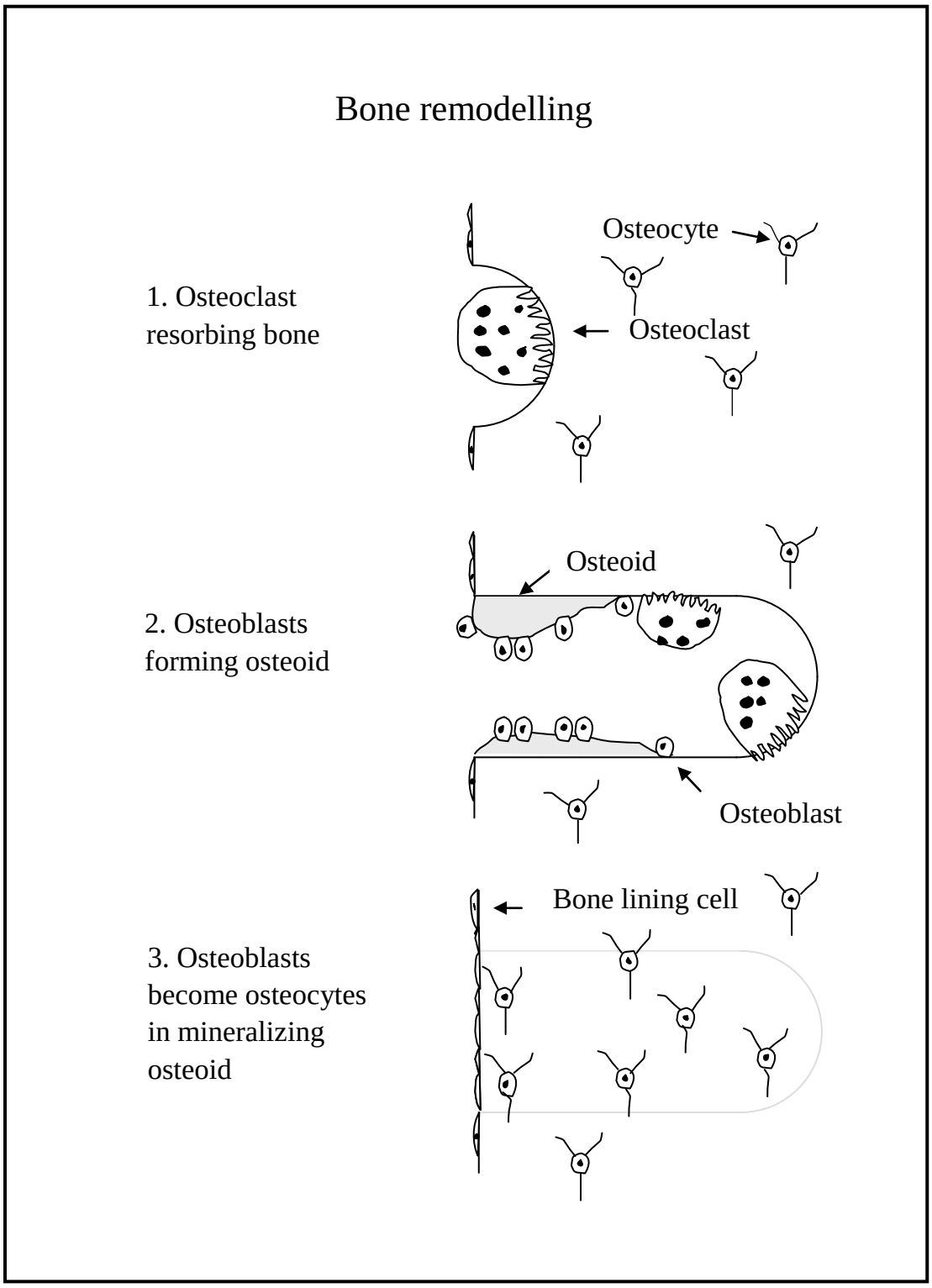


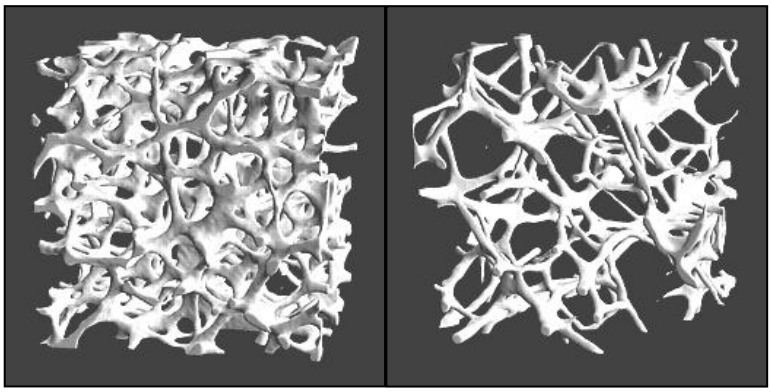
Figure 1. Schematic presentation of bone remodelling.

Bone remodelling and markers of bone turn over

The bone remodelling cycle takes place at fixed sites called a bone remodelling unit (BMU) and begins with osteoclasts resorbing bone by releasing enzymes. Cathepsin K, acid phosphatases and metalloproteinases are released through the osteoclast´s lower, ruffled, boarder. The bone is demineralised and bone matrix proteins subsequently degraded giving rise to a resorption pit in the bone. In this process different degradation products like carboxyterminal telopeptide of type I collagen (ICTP) are released and can be measured in serum as a marker of bone resorption. Other degradation products as well as osteoclast enzymes can also be measured and used as bone resorption markers. When bone resorption is completed after approximately 10 days, bone formation and mineralisation follows. The whole remodelling process takes up to 3 months. During the bone formation phase, osteoblasts synthesise osteoid containing a precursor to collagen type I. The aminoterminal propeptide (PINP) and the carboxyterminal propeptide (PICP) are cleaved during maturation of collagen. These and other markers like bone-specific alkaline phosphatase and osteocalcin, can be measured in serum and reflect bone formation [49-51].

Osteoporosis

Bone strength or structure is hard to measure in vivo, but bone mass can be measured by densitometry techniques. Osteoporosis is a systemic skeletal disease characterised by low bone mass and micro architectural deterioration of bone tissue resulting in increased risk for fractures.



Normal and osteoporotic trabecular bone.

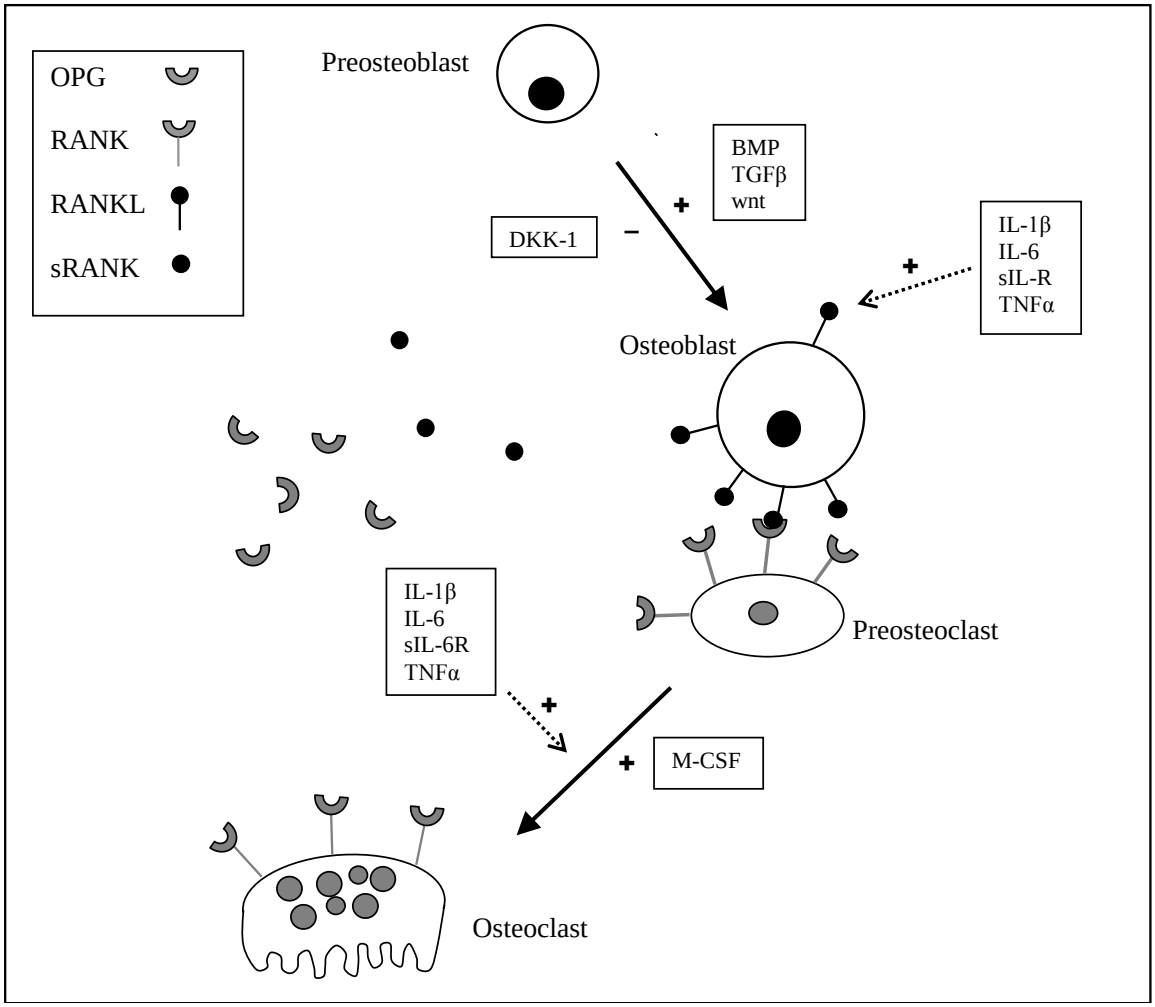
Osteoporosis was defined according to World Health Organisation (WHO) as a bone mineral density (BMD) value at or below -2,5 SD, and osteopenia between -1 and -2,5 SD, compared to the young adult mean value measured at any site using dual x-ray absorptiometry (DXA) [52]. DXA is a method where x-rays of two different energy levels are used. The BMD is calculated after correction for other tissues. There are however some pitfalls. A skeleton with low calcification, not due to osteoporosis but to osteomalacia, will give a low BMD measurement and osteoarthritis or osteosynthetic material results in false high BMD. DXA measures bone as one entity while other methods like quantitative computed tomography (QCT) can distinguish between cortical and trabecular bone [53]. The method in routine clinical use is however DXA. Low BMD does not cause any symptoms and the clinical implication of osteoporosis is the increased risk of skeleton fractures. Osteoporosis can be primary; caused by aging, menopause and life style factors like smoking, alcohol, low physical activity, low sunlight exposure and insufficient intake of calcium or secondary; caused by diseases or medication, especially glucocorticosteroids [54]. It is known that markers of inflammation in rheumatoid arthritis (RA) are associated to local joint bone loss and to generalised osteoporosis, indicating a common mechanism [55] [56]. Hence more inflammation and arthritis caused by withdrawal of glucocorticosteroids resulted in aggravated general loss of bone [57].

Bone and inflammation

Differentiation of the osteoclast progenitor (OCP) and function and survival of the mature osteoclast requires M-CSF and RANKL that signals via the receptor activator of nuclear factor- κB (RANK) present on OCP and mature osteoclasts. RANKL can be expressed by almost all cell types but in bone and immune system by osteoblasts/stroma cells, fibroblast-like synoviocytes and activated T-cells and B-cells [58]. RANKL is cell-surface bound but can be cleaved off [59]. Interaction between osteoblasts and OCP are principally cell-cell interactions [59, 60]. The action of RANKL can be inhibited by osteoprotegrin (OPG), a soluble decoy receptor that compete with RANK in binding to RANKL. OPG down regulate osteoclastogenesis and the activation of mature osteoclasts [58]. OPG is produced by osteoblasts/ stroma cells in response to cytokines and anabolic agents like estradiol and BMPs [61, 62]. OPG is down regulated by glucocorticosteroids, cyclosporine A and parathyroid hormone (PTH) and expression of OPG decline with age [63-66]. The ratio of RANKL/OPG determines and controls the osteoclast activity. All factors that affect the amount of RANKL or OPG will change the bone homeostasis. Proinflammatory cytokines, interleukine-1β (IL-1β), IL-6, sIL-6R, TNF-α and others, stimulate osteoclast differentiation by mediation of the osteoblasts via up regulation of RANKL [60, 67, 68]. In vitro studies have shown that regulatory T-cells have the capacity to inhibit osteoclast differentiation and function. This inhibition was dependent on cell-cell contact and was not mediated through the RANKL/OPG system [69].

Osteoblasts mature in the stroma of the bone marrow. They migrate toward the bone surface where they secrete osteoid. Wnt proteins are a family of proteins synthesized by a group of "wingless" (Wnt) genes. Wnt signalling through the Wnt/β-catenin pathway increases osteoblastogenesis through stimulation of pre-osteoblast replication and inhibition of osteoblast and osteocyte apoptosis [46]. Apocrine Wnt signalling in prostatic tumour cells have been shown to induce bone metastases [70]. Wnt signalling is modulated by the natural inhibitor Dickkopf-1 (DKK-1) [71]. Glucocorticosteroids has been shown to induce DKK-1 expression in osteoblasts, thus inhibiting bone formation [72]. Figure 2.

Figure 2. Schematic representation of bone cell development and the influence of inflammation.



Osteoporosis in SLE

Some risk factors of osteoporosis are of special interest in SLE. SLE affects predominantly women and women have increased risk of osteoporosis compared to men. It has been shown that women with SLE have an earlier menopause than women in general [73]. Sun avoidance is recommended in SLE and is associated with risk of deficiency in vitamin D necessary for bone formation [74]. Possibly physical activity in SLE patients is lower because of arthralgias and constitutional symptoms. It has recently been shown that aerobic capacity is impaired in women with SLE regardless of disease activity or disease damage [75]. Glucocorticosteroids, known to induce osteoporosis, is frequently used in SLE. Regardless of geographic origin of studies, prevalence of current usage of glucocorticosteroids often exceeds 50% and ever taking them differs between 60-98% in cross sectional studies. Other medications with possible side effects on BMD are cytotoxic substances and immunosuppressants used in treatment of SLE flares. Specific trials are rare but one study found BMD to be uninfluenced by intravenous cyclophosphamide during follow up time of 2 years [76]. However cyclophosphamide can induce menopause [73] which enhance BMD loss.

Several studies have demonstrated that low BMD and osteoporosis is more common in SLE than in general population [77-80 81] (I) . It has also been shown that already premenopausal patients have reduced BMD [77] but this was barely found in a Chinese study with low prevalence of osteoporosis [82]. There is no controversy that general risk factors like high age and being postmenopausal also apply for SLE patients. There are however different findings regarding the role of glucocorticosteroids and SLE inflammation with respect to influence on bone. Which of these factors that have the largest impact on BMD is an intricate question since glucocorticosteroids is generally used as medication against SLE inflammation. Several studies have shown lower BMD in SLE patients using glucocorticosteroids [80, 83-85] and that premenopausal women are especially affected [80]. The cumulative dose of glucocorticosteroids is associated to decreased BMD [86]. There might be a threshold mean daily doses of at least 7,5 mg prednisolone taken during 2-3 years to affect BMD [84 87], or ever taking doses of at least 10 mg [88]. Other studies find a weak association between BMD and glucocorticosteroids [76] or no association to current dose [78, 79, 81, 89] (I) or cumulative dose[77, 78, 81, 89, 90] (I). In one study BMD was not lower in 38 patients on long term glucocorticosteroid therapy than in controls, but there was an increase in biochemical markers of bone turnover [91], table 4. Influence on BMD by disease damage is also shown in several studies [81, 85, 90, 92] (I) and association to disease activity have been shown in some cross sectional studies. However disease activity should be prolonged to be able to give measurable lower BMD and is therefore best studied in longitudinal studies. Disease duration is frequently associated to BMD. In all patients disease duration naturally increases by age. Long disease duration could even include patients passing from pre- to post-menopause. Therefore it is especially interesting that

osteoporosis in juvenile SLE patients also is associated with longer disease duration [93], figure 3.

Figure 3. Risk factors of osteoporosis. Roman numerals refer to the papers in this thesis.

There are factors, apart from the usual treatments of osteoporosis, which are associated with maintenance of BMD in SLE. Regular exercise was protective of femoral neck BMD loss [81, 84] (I) and usage of hydroxychloroquine preserved BMD in hip [85] and spine [85, 94]. Taken together there are indications that ordinary osteoporotic risk factors, glucocorticosteroids and inflammation interact and contribute to osteoporosis in SLE patients. Glucocorticosteroids may diminish negative effects on bone caused by SLE inflammation if used in correct doses and in the right patients.

Table 4. BMD in SLE and glucococorticosteroids as a risk factor for low BMD.

Reference	Study design	Patient number	Female %	Age mean±SD, if not indicated otherwise	Glucocorticosteroids risk factor for low BMD Yes (+), No (-)	BMD SLE vs. controls
Kalla [79] (1993)	Cross sectional	46 SLE 108 controls	100%	31±7 32±8	-	SLE < controls
Formiga [77] (1995)	Cross sectional	74 SLE 50 controls	100%	30,8±6,5 30,8±6,9	-	SLE < controls
Kipen [95] (1997)	Cross sectional	97	100%	44,2±14,9	+	
Hansen [76] (1998)	Longitudinal 2 years	36	86%	Median (range) 39 (28-53)	(+)	
Li [82] (1998)	Cross sectional	52 SLE 52 controls	100%	34,1±8,0 33,7±7,7	(+)	
Kipen [84] (1999)	Longitudinal 3 years	32	100%	Mean (SEM) 35,2 (1,5)	Lumbar spine loss if prednisolone ≥ 7,5mg	
Sinigaglia [83] (1999)	Cross sectional	84	100%	30,5±7,5	+	
Gilboe [80] (2000)	Cross sectional	75 SLE 75 RA 75 controls	88%	Median (range) 45 (20-70) Median (range) 45 (22-70) Median (range) 45 (20-70)	+	SLE < controls SLE and RA similar
Jardinet [87] (2000)	Longitudinal 2 years	35	100%	30±9	Lumbar bone loss if prednisolone > 7,5 mg	
Becker [92] (2001)	Consecutively	64	52%	Female: 33±9,2 Male: 36±11,1	+	
Lakshaminarayanan [85] (2001)	Consecutively	92	100%	45,9±12,4	+	
Coimbra [78] (2003)	Cross sectional	60 SLE 64 controls	100%	32,8±8,6 31,1±7,2	-	SLE < controls

Korczowska [91] (2003)	Cross sectional	38 SLE 160 controls	100%		-	SLE = controls
Uaratanawong [86] (2003)	Cross sectional	118	100%	Corticosteroids 31,8±8,1 No Corticosteroids 34,0±7,9	+	
Bultink [89] (2005)	Consecutively	107	93%	41±13	-	
Mok [94] (2005)	Cross sectional	34	100%	52,9±4,9	Not evaluable.	
Yee [88] (2005)	Cross sectional	242	95,5%	Median (range) 40 (18-80)	Prednisolone > 10 mg/day	
Lee [90] (2006)	Cross sectional	307	100%	41,7±11,1	-	
Almehed [81](I) (2007)	Cross sectional	163	100%	Median (range) 47 (20-82)	-	SLE< calculated control value

Vertebral fractures

The mean 10-year probability of getting a vertebral fracture in general female Swedish population has been estimated to 7,2% at 50 years of age, increasing to 26,7% at 85 years of age [96]. An existing, prevalent, vertebral fracture is a risk factor of new fractures and of mortality [97 98]. There is a small risk of getting vertebral fractures in spite of normal BMD [96] although low energy fractures like vertebral are typical osteoporotic fractures. Vertebral compression fractures can occur almost spontaneously in a fragile skeleton. Since fracture symptoms can be hard to distinguish from other causes of back pain, prevalence of vertebral fracture will differ depending on if radiographic or clinical fractures are assessed. The vertebra consists mainly of trabecular bone which is surrounded by a thin cortical layer. Events influencing trabecular strength or spinal mechanical loading will facilitate vertebral compressions. There are different methods of analysing conventional radiographs for fractures. These include semiquantitative and quantitative morphometric methods which have been found equivalent if performed by skilled radiologists [99]. Figure 4.

Higher prevalence of vertebral fractures have been found in female SLE patients than in age matched controls [100-102] and already in premenopausal ages [100]. A high proportion of women suffered from vertebral fractures in spite of normal BMD [103] (II). Risk factors of fracture in SLE were high age, postmenopausality or disease duration [88, 89, 102, 103] (II), low BMD [88, 89, 100], vitamin D deficiency [89] and glucocorticosteroid medication [89, 102]. In some studies associations between fractures and glucocorticosteroids were analysed but not found [88, 100, 103](II). Table 5.

Comparisons of female post-menopausal patients on chronic glucocorticosteroid therapy but with different diseases reveal differences in prevalence of vertebral fractures [104]. SLE patients had fewer fractures than patients with several other inflammatory diseases. It is also known that fracture risk is higher with glucocorticosteroid treatment than without at a given BMD [105]. Taken together, the impact of glucocorticosteroids on vertebral fractures in SLE is still uncertain. Estrogen deficiency has known effects on bone metabolism resulting in net bone loss and lower BMD. Estrogen deficiency in general female population has also been proposed to effect connective tissue and intervertebral disc components leading to reduced disc height and loss of shock-absorbing properties [106]. Female SLE patients have earlier menopauses than healthy women which could have some relevance when comparing fracture prevalences [73]. Vitamin D deficiency increases serum PTH which leads to increased bone resorption and osteoporosis. Severe Vitamin D deficiency causes osteomalacia where new bone matrix, osteoid, is not mineralized resulting in soft bone and sometimes fractures [74]. Several risk factors for fractures apply specially to SLE patients and should be recognised. These factors mainly agree with the risk factors for osteoporosis, figure 3, but include factors increasing the risk of falling like sight

reduction and alcohol use. Vertebral fractures seldom come to clinical attention (II) and therefore spine radiograph should be liberally considered in SLE patients.

Non-vertebral fractures and SLE

Self-reported fractures (vertebral and non-vertebral) were five times more common in SLE patients compared to general population in a large population-based American study. High age and long term treatment with glucocorticosteroids were associated with fracture [102]. In cross sectional studies longer duration of SLE [103] and low BMD was associated with fractures [88, 103].

Figure 4. Semiquantitative visual grading of vertebral deformities [107]. Graphic representation.

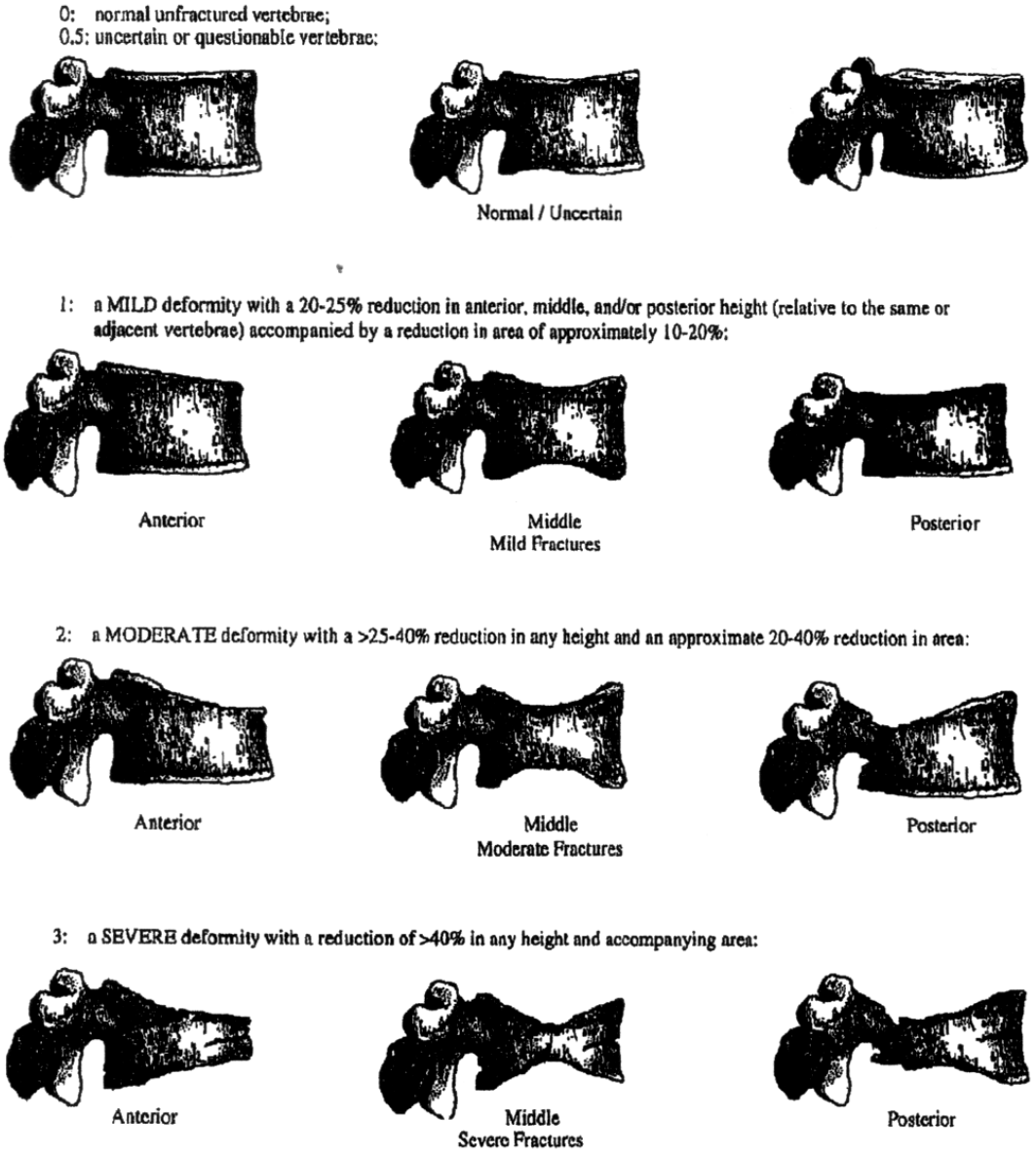


Table 5. Fractures and risk factors of fracture in SLE.

Reference	Study design	Patient number	Female %	Age mean ± SD, if not indicated otherwise	Vertebral fractur (V) Peripheral fracture (PF) Clinical fracture (CF) Radiological fracture (RF)	Percentage of fractures, SLE vs. control	Glucocorticosteroids risk factor for fracture: Yes (+), No (-) Low BMD risk factor for fracture: Yes (+), No (-)
Ramsey-Goldman [102] (1999)	Retrospective cohort	702 SLE US population controls (NHIS)	100%	45,4± 13,1	V, PF, CF	SLE > control	Glucocorticosteroids : + Low BMD: Not assessed
Yee [88] (2005)	Cross sectional	242	95,5%	Median (range) 39,9 (18-80)	V, PF, CF		Glucocorticosteroids : - Low BMD: +
Borba [100] (2005)	Cross sectional	70 SLE 20 controls	100%	31,8±8,1 32±8,9	V, RF	SLE > controls	Glucocorticosteroids : - Low BMD: -
Bultink [89] (2005)	Consecutively	107	93%	41± 13	V, RF		Glucocorticosteroids : + Low BMD: -
Lee [103] (2007)	Cross sectional	307	100%	41,7± 11,1	CF		Glucocorticosteroids : - Low BMD: -
Rhew [101] (2008)	Longitudinal 2 years	100 SLE 100 controls	100%	44,1± 11,1 44,5± 10,7	V, PF, CF	SLE > controls (incident nonvertebral fractures)	Glucocorticosteroids : - Low BMD: -
Almehed (II)	Cross sectional	163	100%	Median (range) 47 (20-82)	V, RF		Glucocorticosteroids : - Low BMD: -

NHIS: National Health Interview Survey

Health related quality of life in SLE

SF-36

Health was defined by WHO as "a state of complete physical, mental and social well being and not merely the absence of disease or infirmity" [108]. Health is a subjective judgement of greatest importance to the patient but without obvious disease correlates. There are several assessments for different aspects of health and quality of life. Some, like the Health Assessment Questionnaire (HAQ), are developed as disease specific assessments while others, for example Medical Outcome Study Short Form-36 (SF-36), is a generic instrument of physical and mental components of self-reported health related quality of life (HRQOL) [109-111]. The concepts of SF-36 are not specific to any age or disease which for example allows comparisons between patient groups with different diseases. This property and the possibility of digital processing have made SF-36 widely used. SF-36 is a validated 36 item questionnaire comprising 8 domains of physical and mental health. The physical domain consists of physical functioning (PF), role limitations due to physical problems (RP), bodily pain (BP), general health (GH) and the mental domains consist of vitality or energy level (VT), social functioning (SF), role limitations due to emotional problems (RE) and mental health (MH). The questions refer to the previous month. SF-36 has been validated in a Swedish version [110]and in SLE [112].

Factors with impact on HRQOL in SLE

HRQOL is scored worse, multidimensional, by SLE patients compared to matched controls [113-115] (III). SLE patients score HRQOL better (higher) than RA patients, especially with regard to physical function [114, 115]. In one study which used the Quality of life scale (QOLS-S) there was no difference between SLE and RA patients, but RA patients scored worse in pain score than SLE patients using Arthritis Impact Measurement Scales (AIMS) [116]. In comparison to patients with other chronic illnesses like congestive heart failure or former myocardial infarction, SLE affect all health domains in SF-36 more, and at earlier ages [117]. However when compared to patients with fibromyalgia, SLE patients score higher (better) in several dimensions of HRQOL. Fibromyalgia is one contributor to worse HRQOL in SLE [118, 119] as is depression and anxiety [120]. Fatigue and the unpredictable course of the disease, the loss of control over the body, were areas especially mentioned by SLE patients as influencing the quality of life in a Swedish study[121]. SLE disease activity and damage scores are often regarded as poor indicators of HRQOL since results regarding associations to SF-36 are not uniform [122] (III). However health status measures correlated better to disease damage index than disease activity indicating that some health measures can capture the consequences of disease over time [114]. In female postmenopausal population with osteoporosis (but without SLE), vertebral fractures contributed significantly to low HRQOL, especially if the fracture was located in lumbar spine [123, 124]. In SLE, prevalent vertebral fractures were only associated with PF

which was significantly lower than in patients without fracture (III). However few patients had fractures in lumbar spine.

HRQOL and work in SLE

Employment rates in SLE are not only related to disease specific factors but also to basic socioeconomic conditions, general employment rates and the present disability pension system. Several factors apart from SLE also influence patients HRQOL. This must be remembered when interpreting study data. In a small Swedish unselected SLE population two years disease did not influence employment rates from that in normal population [125], but absence due to sickness was common. In an American study with 900 working SLE patients included, the proportion of employed patients after 5 years disease was approximately 85% and after 10 years 60% [126]. In a Dutch study with 59% unemployment rate reduced HRQOL, higher age at disease onset, neuropsychiatric organ damage and diabetes were associated with unemployment in SLE. In an American study with 43% work-disability among SLE patients disease damage, pain and fatigue was associated with work-disability [127]. In a Swedish study with 42% unemployment, 40% work disability, patients working scored higher in all SF-36 subscales than patients with work-disability. Low age and high PF and RP were associated with the capacity to work (III).

Factors improving HRQOL in SLE

Several factors are known to be associated with HRQOL but few studies have looked at the possibility of specific intervention to improve HRQOL in SLE. Improved physical health has however been associated with better coping strategies and improved mental health. Improved mental health has been associated with better family support, lower disease activity and glucocorticosteroid dose, non-use of cytotoxic drugs and better physical health [128]. Thus a potentially modifiable factor in SLE treatment, apart from optimized medical treatment of the disease itself, is education of the patients and their families aiming at better coping strategies and support.

Inflammatory markers in SLE

Since SLE includes diverse disease manifestations of different severity, there is need to identify specific biomarkers of SLE activity. Up today disease activity measure is mainly performed with assessments like SLEDAI, BILAG or SLAM. There is ongoing search for more specific markers of disease activity, inflammation, and prediction of SLE flares. In the T helper cell (Th) Th1/Th2 model, cytokines produced by CD4+ T helper cells are functionally grouped in Th1 and Th2 cytokines. Th1 cytokines mainly induces cellular immunity; IL-2, IL-12, interferon (IFN)-γ and Th2 cytokines mainly induces humoral immunity and antibody production; IL-4, IL-5, IL-6 and IL10 [129, 130]. An uneven balance in Th cytokine production could favour the development of certain diseases in susceptible individuals. Dominance of Th 2 cytokines has been shown in SLE [131] although results are not totally consistent [132]. Measurement of interleukins have however not so far been of great clinical value, with the exception of measurement in cerebrospinal fluid in CNS lupus [133]. Complement factors, especially factors early in the classical activation pathway, are of clinical interest in lupus [134]. Antibodies against C1q are found in autoimmune diseases like SLE, sometimes in infections, and are linked to immune-complex disorders. The physiological role of C1q is clearance of immune complexes and apoptotic cells from the organism. In SLE C1q deficiency is associated with nephritis [135, 136]. Decrease in C3 and C4 have also been described prior to and in SLE flares, mainly in kidney and haematological affection [137] [138]. Together with decreasing complement levels rising concentrations of anti-dsDNA in serum has been shown to predict major exacerbations [138, 139].

Resistin

Resistin is a low molecular weight, cystein rich secretory peptide discovered some years ago by three different groups [140-142]. In rodents resistin is mainly produced in white adipose tissue and may be the linkage between obesity and insulin resistance. There is however only a 59% homology at protein level between resistin in mouse and in humans, and the genes are coded on different chromosomes [143]. In man resistin is expressed mainly in macrophages and other myeloic cells [144] but also in osteoblasts and osteoclasts [145] and several other tissues [146]. In general population resistin is higher in females than in males and associated with elevated CRP or IL-6 [147, 148]. Resistin levels are higher in patients with impaired renal function [149-151] but this relation is not present in normal or mildly impaired function [152]. Resistin has been associated with vascular inflammatory markers in type 2 diabetes mellitus [153], with prolonged inflammation during sepsis [154], with inflammation in obstructive sleep apnea [155], Crohn´s disease [156], RA [157-159], local salivary gland lymphocytic inflammation in Sjögren´s Syndrome [160] and with inflammation and glucocorticosteroid medication in SLE (IV).

In vitro studies on peripheral blood mononuclear cells show that resistin increase mRNA expression of IL1, IL-6 and TNFα [159]. In addition, resistin expression is up regulated by the same cytokines [161]. Resistin has been shown to stimulate osteoclastogenesis in vitro via NFκB pathway, possibly via osteoblast IL-6 secretion [145] indicating a role for resistin in bone remodelling. In general male population no clear-cut association between resistin and BMD has been demonstrated [162, 163]. In RA association was found between resistin and low BMD [158] and this was also shown in SLE (IV). Resistin could be a key cytokine in SLE inflammation. There is need for disease mechanisms explaining both general inflammation and long term disease complications like osteoporosis and cardiovascular disease. The role of resistin in humans with regard to insulin resistance and possibly vascular disease is not as clear as in rodents. However data indicate interplay between inflammation, resistin and endothelial dysfunction [164-166]. This would make resistin a perfect target for medical intervention in SLE, influencing both inflammation and cardiovascular complications. We therefore look forward to further studies regarding resistin and possible mechanisms of action in general and in SLE.

Patients and methods

Patients

Three hundred thirty-nine patients, men and women, with SLE were identified from the patient administrative registers in the rheumatology clinics in Göteborg and Borås. The patients were invited to participate in a cross sectional study investigating; Paper I. Frequency and determinants of osteoporosis in SLE. Paper II. Frequency and determinants of vertebral fractures in SLE. Paper III. Health related quality of life in SLE. Paper IV. Possible important markers of osteoporosis and/or inflammation in SLE.

All patients completing the study were at least 18 years old and fulfilled at least four of the 1982 American Collage of Rheumatology (ACR) classification criteria for SLE [3]. Exclusion criteria were pregnancy and not speaking Swedish.

One hundred eighty-two patients, 163 women and 19 men, completed the survey. The procedure of enrolment to the study and reasons for discontinuation are shown in figure 5. Only results from female participants are analyzed. In Paper I, II and IV results from 163 female SLE patients are shown. In Paper III 150 female patients are analyzed. Thirteen of the patients were excluded in this analysis because of lacking radiographs of thoracic and lumbar spine, one examination was not performed and twelve analogue radiographic pictures were damaged by a flooding of the archive.

All patients gave informed written consent prior to participation and the study was approved of by the Ethics Committee at Sahlgrenska Academy at University of Gothenburg.

Controls

Paper I. A control BMD was calculated for each patient using the equations for Z-score estimations from the Lunar Prodigy (12165) software, provided by GE Healthcare. These adjust the BMD values for age and weight [167].

Paper III. An age and sex-matched reference group (n=1045) was randomly selected from the Swedish SF-36 national normative database (n=8930).

Paper IV. The control group consisted of 12 female healthy blood donors and 30 healthy female staff members and PhD students in the department of Rheumatology.

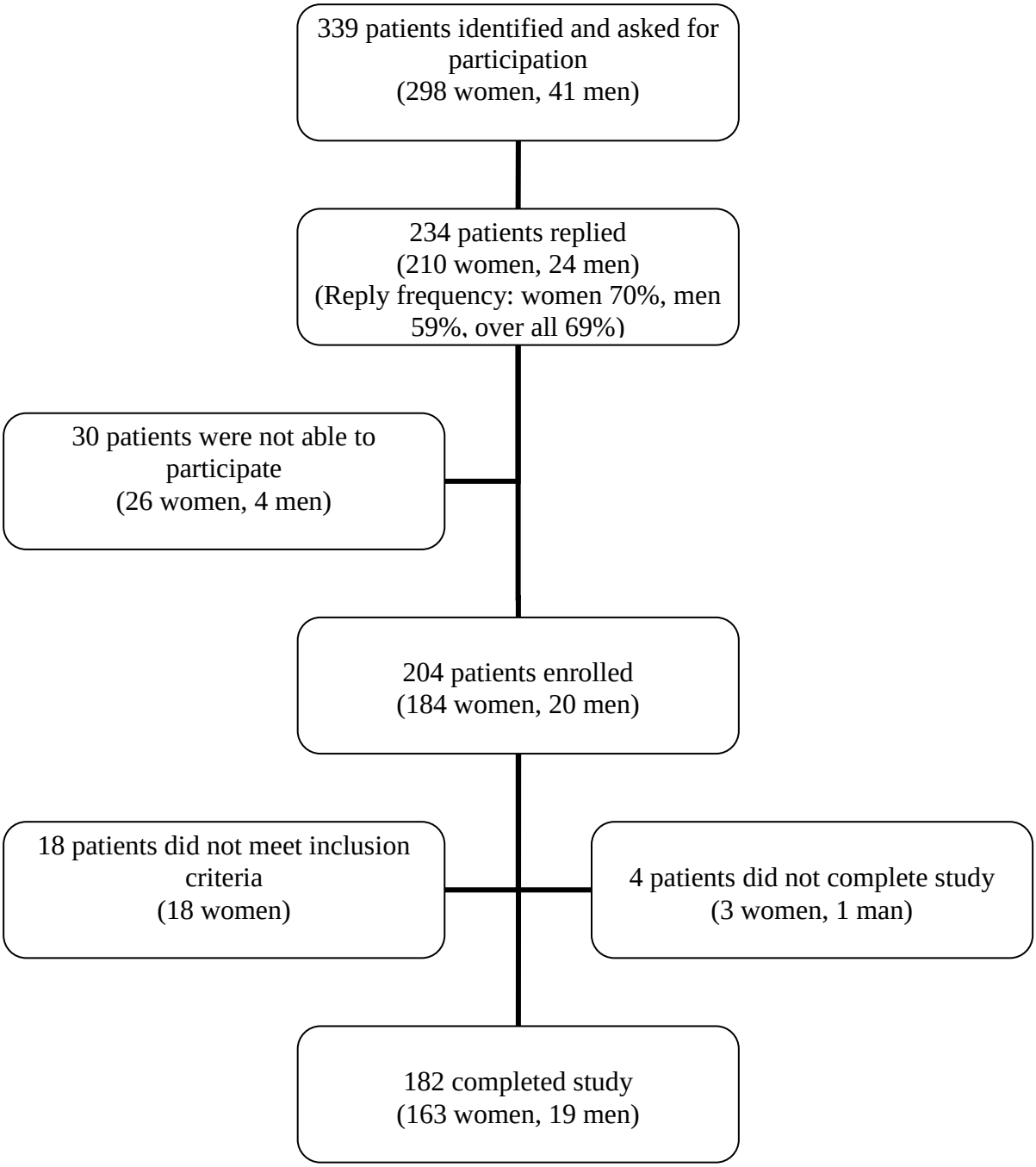


Figure 5. Procedure of enrolment to study and reasons for discontinuation.

Questionnaire

Paper I-IV. All patients answered self administered questionnaires about age, duration of disease, medication, intake of cheese and milk, smoking habits, physical activity, menopausal status, fractures obtained after 25 years of age and current mean of livelihood. DMARD medication was recorded as either a single treatment or combinations of two or three DMARDs. Dietary calcium intake was calculated from information on average intake of cheese and milk.

Cumulative corticosteroid intake was calculated as thoroughly as possible by reading all patients medical records.

Assessment of disease activity, disease damage and quality of life

Paper I-IV. Disease activity was scored by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI-2K) [41] and disease damage was recorded according to Systemic Lupus International Collaborative Clinics (SLICC/ACR) [44]. Health related quality of life was assessed by the Swedish version of the Medical Outcome Study Short Form-36 (SF-36) [168] , also validated in patients with SLE [112]. SF-36 was the questionnaire first answered by each patient.

Laboratory methods

Paper I-IV. Venous blood samples were obtained in the morning after an overnight fast and serum was stored at -70°C until analysis. ESR, CRP, creatinine, blood cell count, ionized calcium, total cholesterol, HDL, LDL, triglycerides and complement factor C3 and C4 were measured consecutively using standard laboratory techniques. Urinary samples were also analysed consecutively.

Quantitative sandwich ELISA kits were used for measurement of proinflammatory cytokines TNFα, IL1β, IL6 and sIL6R (Quantikine, R& D Systems, Minneapolis, USA).

The bone resorption marker, C-terminal telopeptide of type I collagen (ICTP), and the bone formation marker, N-terminal propeptide of type I procollagen (PINP), were analysed quantitatively in serum by radioimmunoassay (RIA) (Orion Diagnostica, Espoo, Finland). Resistin levels were detected with a sandwich ELISA (R & D Systems, Minneapolis, USA). The detection limit of the assays were: TNFα 0,12 pg/ml , IL1β 0,1 pg/ml, IL6 0,7 pg/ml and sIL6R 6,5 pg/ml, ICTP 0,7 µg/l, PINP 2 µg/l and resistin 31 pg/ml.

Bone mineral density

Paper I-IV. BMD was measured at the lumbar spine (L2-L4), non dominant hip (femoral neck and total hip) and non dominant distal forearm (the diaphysal 33% of radius and total radius) using a Lunar Prodigy densitometer, 12165 (GE Medical Systems) . The precisions for duplicate measurements were 0,9% for lumbar spine, 0,5% for left total hip and femoral neck and 2,8% for radius. All BMD results were expressed in absolute values (g/cm^2) and as the number of standard deviations (SD) above or below the mean results of young female adults, T-score, and compared to an age matched female reference population consisting of approximately 12000 healthy women aged 20-80 in a pooled North European/ United States population [167], Z-score. Osteoporosis was defined according to World Health Organisation (WHO) as a BMD

value at or below -2,5 SD, and osteopenia between -1 and -2,5 SD, compared to the young adult mean value measured at any site using DXA [52].

Vertebral fractures

Paper II-IV. Each patient had two lateral conventional radiographs, one of thoracic spine and one of lumbar spine, taken. The vertebras were visually graded by Genant´s method [107] as normal (grade 0), mildly deformed (grade 1, approximately 20-25% reduction in anterior, middle and/or posterior height and a 10-20% reduction in area), moderately deformed (grade 2, approximately 25-40% reduction of any vertebral height and a reduction in area of 20-40%), and severely deformed (grade 3, approximately 40% reduction in any vertebral height and area). A vertebrae graded 1, 2 or 3 was regarded as fractured. One radiologist (S.H) evaluated all radiographs.

Statistical analysis

In paper I Pearson´s Correlation was used in simple regression analyses with BMD or cumulative corticosteroid dose as dependent variables and demographic and disease-related variables as independent variables. In paper II-IV all variables were tested with Kolmogorov-Smirnov ´s normality test. Pearson´s Correlation was used when variables were normally distributed otherwise Spearman´s rank Correlation was used. Unpaired *t*-test was used to compare numeric data (paper I-IV). One example is when we compared BMD-values between pre- and post-menopausal women (paper I). Mann-Whitney U-test was used for comparisons of not normally distributed variables between patients with or without vertebral (paper II). Categorical data have been compared with χ^2-test (paper I-IV).

In paper I observed BMD-values were compared with each patient's expected BMD-value by sign test. Multiple regression analyses, forward stepwise method, have been performed to explore the relationship between BMD in different sites and demographic and disease-related variables (paper I). The same method was used with PCS as dependent variable in paper III and with resistin as dependent variable in paper IV. Independent variables were demographic and disease-related variables that had shown significant correlations in simple regression. Logistic regression, forward conditional method, was used to explore relationships between a categorical dependent variable and covariates (paper II-IV**).** The significance of the logistic regression analyses was expressed by calculating an area under ROC curve with confidence interval (paper II-IV). In paper IV correlations between serum resistin levels and ages in SLE-patients and controls are compared. The constant and the regression coefficient, with respect to resistin values and age, were compared to the corresponding parameters of the controls by use of a special t-test. All tests were two-tailed and $p<0,05$ was considered statistically significant.

Main conclusions from the thesis

Paper I

BMD in female SLE patients was compared to reference values of BMD. The reference BMD for each patient was calculated from equations derived from the DXA adjusting it for age and weight. Risk factors associated to low BMD were calculated. We found that the SLE patients had significantly lower BMD than the healthy references in all measured sites. Patients fulfilling our present criteria for treatment against low BMD, either osteoporosis or osteopenia and glucocorticosteroid medication, were seldom treated adequately. Risk factors of low BMD were known factors like high age and low weight but also markers of inflammation, impaired renal function and disease damage. These results show the need of better awareness of osteoporosis and adequate treatment of low BMD. Since systemic inflammation in SLE is associated to low BMD we hypothesis that glucocorticosteroid medication by suppression of the systemic SLE inflammation in some circumstances, could preserve BMD.

Paper II

Low BMD is one of several known risk factors of fragility fractures. In this study we analysed the prevalence of vertebral fractures in SLE. We also wanted to establish possible SLE-specific factors associated to vertebral fractures in our patient cohort. The results show that vertebral fractures are frequent but they are seldom clinically diagnosed. Although 52% of the patients were on medication with glucocorticosteroids, there was no association between treatment with glucocorticosteroids and vertebral fracture in this study. We found no independent correlation between SLE-specific factors and vertebral fractures. High age was the most important factor associated with vertebral fracture. A high percentage (40%) of patients with vertebral fractures had normal BMD in all measured sites indicating the importance of other biomechanical properties then BMD for bone strength. Until disease specific factors affecting bone strength are found, established risk factors of osteoporosis and fractures in general population must be used to guide the clinician to select SLE patients for x-ray.

Paper III

Health quality is a subjective experience not necessarily dependent on having a disease. In this study we analysed self-reported physical and mental HRQOL and its relationship to disease variables, vertebral fractures and employment status. The SLE patients scored significantly lower HRQOL in all measured health domains compared to age and sex matched controls. We found associations between physical component summary score (PCS) and BMI, disease activity, glucocorticosteroid dose, age and SLICC. When patients 20-64 years old were analysed, working capacity was highly

significantly associated with PCS. Patients working full or part time rated all measured health domains, both physical and mental, higher than patients not working. There was no difference in disease activity, DMARD treatment or prevalence of vertebral fractures between the groups although patients working were younger and had shorter disease duration and lower SLICC. When aiming at better HRQOL in SLE, factors of importance to working ability should be given special attention.

Paper IV

Resistin is a recently described secretory peptide with proinflammatory properties in humans. We investigated relations between resistin and other markers of inflammation, kidney function and BMD in SLE. Serum levels of resistin measurements did not differ between patients and healthy controls, possibly reflecting that we measure the spill-over from local tissue compartments in the circulation. In spite of this, resistin was associated to inflammation, renal function, glucocorticosteroid treatment and BMD. Resistin is a marker of inflammation but we do not know if it has pathophysiological significance in SLE. We suggest that resistin can be a linkage between inflammation and deteriorating bone quality in SLE. Further studies are encouraged.

Popularized summery in Swedish (Populärvetenskaplig sammanfattning)

Systemisk Lupus Erythematosus (SLE) är en autoimmun sjukdom som till övervägande del drabbar kvinnor. Av patienterna är 9 av 10 kvinnor och de flesta insjuknar i fertil ålder. Sjukdomen är vanligare i svart och asiatisk befolkning än bland kaukasier. I Sverige har ungefär 68 av 100 000 invånare SLE medan det årligen nyinsjuknar 4-5 personer per 100 000 invånare.

Sjukdomen är multifaktoriell vilket vid SLE betyder att såväl genetiska som hormonella och miljöfaktorer är betydelsefulla för utveckling av sjukdomen. De gener som är av betydelse hör samman med immunsystemet. Solljus är en miljöfaktor som har visats kunna försämra sjukdomen liksom det kvinnliga könshormonet östrogen.

De flesta SLE patienter har autoantikroppar mot beståndsdelar i cellkärnan (ANA) och mot arvsmassan (anti-DNA). Det kan också finnas andra autoantikroppar. Många har inflammation i huden med hudutslag, smärta och svullnad i leder eller sår i munslemhinnan. Det kan förekomma utgjutning i lungsäck och hjärtsäck samt inflammation i njurar och centrala nervsystemet. Inflammationen vid SLE behandlas med immun- och inflammationsdämpande mediciner bl.a. kortison och ibland cellgifter.

Benskörhet eller osteoporos är ett tillstånd i skelettet när benmineraltätheten (BMD) är nedsatt. Vid osteoporos är skelettets hållfasthet sänkt och det är lättare att ådra sig benbrott. Ett vanligt brott på skelettet vid osteoporos är kotfrakturen där kotan pressas samman.

Vi har undersökt BMD hos 163 kvinnor med SLE. Undersökningsmetoden är dual energy x-ray absorptiometri (DXA), en form av lågenergiröntgen. Med utgångspunkt från ett referensmaterial omfattande BMD på ca 12 000 personer kunde vi räkna fram hur varje patients BMD ”borde” vara med hänsyn tagen till ålder och vikt. SLE-patienterna hade genomsnittligt lägre BMD än var de borde ha haft. De faktorer som var associerade till låg BMD var tidigare kända allmänna riskfaktorer som hög ålder och låg vikt men även inflammation, sänkt njurfunktion och sjukdomsskada av SLE-sjukdomen var betydelsefulla. Vi fann först ett samband mellan kortisonmedicinering och BMD men det sambandet kvarstod inte när fler faktorer togs med i analysen. Regelbunden fysisk aktivitet var skyddande för skelettet i höfterna. Få patienter med indikation för behandling av osteoporos hade denna medicinering.

Samtliga 163 patienter röntgade också ryggen för bedömning av om det förelåg frakturer i form av sammanpressade kotor, kotkompressioner. I denna del av studien kunde 150 patienter bedömas då 12 röntgenbilder vattenskadats under lagring och en

bild förkommit. Fyrtiotre (29%) av patienterna hade tillsammans 95 kotkompressioner men bara 6 (4%) hade tidigare fått sin kotfraktur diagnostiserad. Det betyder att så många som 25% av hela patientantalet hade minst en kotfraktur utan att veta om det. De flesta frakturerna satt i mellersta bröstryggen. Andelen patienter med kotfraktur steg med ökande ålder. Flera faktorer var associerade med kotfraktur men hög ålder var den viktigaste riskfaktorn. Man vet sedan tidigare att kortisonbehandling leder till ökad risk för låg BMD och frakturer. I denna studie har vi inte funnit dessa samband. Det kan bero på studiens utformning, men det kan också bero på att inflammationen vid SLE i sig är skadlig för skelettet. Kortison i låga doser skulle då kunna minska inflammationen så att nettoeffekten blir positiv för skelettet, BMD bibehålls.

Hälsa är definierat av Världshälsoorganisationen (WHO) som "ett tillstånd av fullständigt fysiskt, psykiskt och socialt välbefinnande, och inte enbart frånvaro av sjukdom eller handikapp”. Olika aspekter på hälsorelaterad livskvalitet (HRQOL) kan mätas med frågeformulär. Vi har använt den svenska versionen av SF-36 som med 36 frågor mäter 4 psykiska och 4 fysiska hälsoområden. Vi frågade också alla patienter om de yrkesarbetade/studerade, var arbetssökande, sjukskrivna, ålders eller sjukpensionärer. I samtliga hälsoområden skattade SLE patienterna sin hälsa som sämre än vad ålders och könsmatchade referenspersoner gjorde. Bland personer 64 år och yngre var låg fysisk hälsa associerad till låg arbetsförmåga, hög SLE aktivitet, hög kortisondos och högt BMI. Inga av de faktorer som vi analyserade var associerade till mental hälsa. Det som kännetecknade en yrkesarbetande patient var yngre ålder och att man uppfattade liten påverkan på två av områdena inom fysisk hälsa (fysisk funktion och rollbegränsning av fysiska orsaker). Denna del av studien visar att HRQOL påverkas av många faktorer förknippade med SLE. Den visar också på en möjlighet att förbättra HRQOL genom förbättrad SLE-behandling såväl som genom påverkan av faktorer förknippade med arbetsoförmåga.

Resistin är ett ämne, en cytokin, som finns i förhöjda nivåer vid flera inflammatoriska sjukdomar och tillstånd. Det är tidigare inte är undersökt om det är så även vid SLE eller om resistin kan associeras till inflammation vid SLE. Nivåerna av resistin i serum skilde sig inte mellan SLE patienterna och de friska kontrollerna. Trots det var resistin associerat till inflammation, sänkt njurfunktion, högre pågående kortisondos, sänkt BMD och lågt high-density lipoprotein (HDL, ”det goda kolesterolet”). Inflammation, sänkningsreaktion, var den faktorn som resistin var oberoende associerat till. Vi tror att resistin kan vara ett ämne som hjälper till att driva inflammationen i SLE och inte bara är en markör för inflammation. Resistin kan också vara betydelsefullt i samspelet mellan benomsättning och inflammation. Det skulle då kunna vara en angreppspunkt för framtida behandlingar. För att säkert kunna veta hur det ligger till behövs fler studier om hur reistinnivåer förändras med patienternas sjukdomsaktivitet. Dessutom behövs studier som belyser hur resistin utövar sin effekt i kroppen.

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