SOLUBLE TUMOR-ASSOCIATED ANTIGENS AND SECONDARY MALIGNANCIES IN AUTOIMMUNE-RHEUMATOID DISEASES

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1. INTRODUCTION

1.1. General information

Autoimmune-rheumatoid diseases often share common components in their pathomechanism, like genetic susceptibility, autoimmune processes induced by certain exogenous factors (e.g. infectious agents, environmental effects, smoking) and the chronic inflammation which – in the absence of proper treatment – may lead to the destruction of multiple organs or tissues.

Present dissertation discusses the current aspects of rheumatoid arthritis (RA), systemic sclerosis (SSc) and systemic lupus erythematosus (SLE).

In short, RA is presented with a chronic multi-joint inflammation. The autoimmune process leads to synovitis and arthritis – most commonly the small, but also the larger joints may be affected, usually in a symmetric pattern. Involvement of the articular cartilage and bone surfaces will ultimately result in structural damage and loss of function.

The pathogenesis of SSc is characterized by inflammation, vascular disorders and tissue fibrosis. These processes lead to fibrosis of the skin (scleroderma) and organic complications, like pulmonary alveolitis and fibrosis, pulmonary hypertension, cardiomyopathy and kidney disease.

In SLE several autoantibodies are produced due to complex disturbances of the immune regulation, impaired immune tolerance and dysfunction of the regulatory T cells. Progression of the autoimmune inflammation may lead to the damage of the affected organs (primarily the skin, kidneys, heart, lungs, liver, joints and nervous system). Most organic alterations are induced by immune complex mediated mechanisms (Type III hypersensitivity).

1.2. Relationship between rheumatoid diseases and malignancies

Rheumatoid diseases are linked to malignant tumors in several ways: a) chronic tissue inflammation increases the incidence of secondary lymphoproliferative diseases and solid tumors; b) long-term immunosuppressive medication may be oncogenic; c) inflamed leukocytes – like the tumor cells themselves - may express tumor antigens which appear in the

serum and may also correlate with disease activity; d) on the other hand, several tumors may mimic autoimmune diseases by presenting with rheumatoid paraneoplastic syndromes (e.g. polyarthritis, scleroderma, myositis, RA or lupus).

This dissertation focuses on secondary malignancies and soluble tumor-associated antigens in autoimmune diseases with occasional references to therapeutic considerations.

1.3. Development of secondary tumors in rheumatoid diseases

During the last two decades, it became evident that the incidence of malignant tumors increased in certain autoimmune-rheumatoid diseases. The chronic immunopathological reaction and inflammation play a crucial role in the development of malignancies (primarily lymphomas but also solid tumors). This effect is contributed to the long-lasting underlying disease but the immunosuppressive medication may also be a risk factor. It is often not easy to make a distinction between these two factors and determine the exact cause of cancer development. It must be noted, that in the past survival rates in autoimmune diseases were much poorer than nowadays and patients did not live long enough for the occurrence of secondary malignancies. These days, however, effective treatment modalities and careful follow-ups yield a 5-year survival over 90% in most autoimmune diseases – a reason for the recent increase in the incidence of secondary tumors.

1.3.1. In RA the frequency of lymphoproliferative tumors was observed to be higher and the incidence of colorectal and ventricular malignancies to be lower than in the average population. This increased rate of lymphoid malignancies may be caused the extensive, chronic B cell stimulation, as is the case in Sjögren's syndrome and SLE. Previously the role of the B cells was not considered fundamental in the pathogenesis of RA; now it is evident that B cells actively take part in the antigen presentation and production of autoantibodies against the rheumatoid factor and citrullinated proteins. Elevated levels of the rheumatoid factor increase the risk of lymphoma development. As far as solid tumors concerned, some studies reported the frequency of bronchial cancer to be higher, while the incidence of colorectal and breast cancers to be on par or somewhat lower, as compared to the general population. It must be noted that the B cell inhibiting treatment (anti-CD20 antibody; Rituximab), recently introduced into the RA therapeutic regime as second-line choice, might

lower the increased lymphoma risk but data are yet to be confirmed. The decreased colorectal carcinoma risk may be contributed the prolonged intake of non-steroid anti-inflammatory drugs (NSAIDs), most importantly the cyclooxygenase-2 inhibitors (COX-2). The overall rate of increase in the risk of malignancies in RA, however, is controversial. On the one hand, autoimmune diseases undoubtedly make the patient more prone to malignancies; also immunosuppressive agents – which may increase the tumor risk on their own – significantly decrease disease activity and progression.

1.3.2. In SSc 3-11% of all cases turn into malignancy. An increase in the incidence of lymphoproliferative diseases has been reported, while solid tumors tend to develop in the organs affected by fibrosis (skin, esophagus, lungs).

1.3.3. In SLE the available data comes from mostly small-scale cohort studies only. Several workgroups observed the increased occurrence of lymphomas, certain sarcomas and breast tumors but their results are not fully confirmed.

1.3.4. Although not related closely to the topic of this dissertation, we briefly mention another autoimmune disease, Sjögren's syndrome, which is particularly important when speaking of secondary malignancies. This disease is relatively frequently associated with RA, SSc and SLE and is an independent risk factor of cancer development. Sjögren's patients face a 13-44x risk increase for developing NHL, making Sjögren's syndrome a fine example for the relationship between chronic autoimmune diseases and secondary malignancies. On the contrary, the incidence of solid tumors in Sjögren's is not higher than in the general population.

1.4. Possible oncogenic effects of immunosuppressive treatment

Treatment regimes for autoimmune-rheumatoid diseases include many cytotoxic drugs. Some of these are known to have direct or indirect carcinogenic properties. Certain drugs damage the DNA in a direct way – these are called mutagens -, eventually leading to an increased risk of cancer. General immune suppression may play a role in the development of EBV-induced lymphomas. Cyclophosphamide has direct tissue toxicity and may cause bladder cancer. Methotrexate or Cyclosporine A has a more favorable side-effect profile.

Prolonged AZA treatment increases the risk for both the solid and hematologic (lymphomas and leukemias) malignancies. The chance of tumor development is correlated with the duration of medical treatment and the cumulative doses, and probably with concomitant smoking habits. In the case of newer drugs (e.g. leflunomide, biologic therapeutic agents) risk assessment has been hardly carried out due to the limited experience with these agents. During the past two decades biologic agents proved a breakthrough in treatment. Several workgroups studied the link between malignant tumors and TNFα inhibitors (infliximab, etanercept and adalimumab). In most studies the frequency of tumors was associated the cumulative dose of the biologic agents.

Interestingly, from the newer drugs the B cell inhibitor anti-CD20 antibody (rituximab) seems to exhibit protective effects against lymphomas. Rituximab was originally developed for the treatment of the B-cell NHL and was only later introduced into the RA treatment regimen. In other B-cell derived diseases – like primary Sjögren's syndrome – the increased cancer risk may be countered by rituximab administration. In RA there is no available study data in this respect.

It must be emphasized that the main risk factor for the development of lymphomas and other secondary malignancies seems to be improperly treated, constantly active autoimmune disease.

1.5. Soluble tumor-associated antigens in rheumatoid diseases

Cancer cells carry tumor-associated antigens (TAA) on their surface which appear in the body fluids in a soluble form. Several TAA-s are presented not only on the tumor cells, but also on activated leukocytes. Those take part in cellular adhesion due to their carbohydrate and protein chains. Elevated serum levels of several TAA-s have been reported in some autoimmune diseases like RA, SSc and SLE.

Probably the best documented TAA family is the carcinoembryonal antigen family (CEA, CD66). Its members are presented dominantly on colorectal and gastric cancer cells. Our workgroup was among the firsts to confirm the presence of the CEA antigens not only on tumor cells, but also on the surface of neutrophil granulocytes and monocytes/macrophages. Acting as adhesion receptors, the CD66 molecules facilitate connection of the tumor cells and leukocytes to the vessel wall thus contributing to metastatic spreading and the progression of inflammatory processes. CD66 antigens can be detected in synovium of patients with RA.

Apart from the CEA family, other TAA-s may also be connected to inflammatory processes and autoimmune-rheumatoid diseases. The CA 15-3 is expressed primarily on tumor cells in breast cancer, CA 19-9 in pancreas cancer, CA 125 in ovarian cancer and CA 72-4 in gastric and mucinous ovarian cancer. These markers also play a role in cell adhesion.

In the case of TAA-s it was suggested that their production correlated with the organ-specific manifestation and activity of the underlying disease. In RA several laboratory parameters can be used as markers of disease activity; the level of the IgM rheumatoid factor (RF) is a good prognostic marker but it does not correlate with clinical activity. CRP can be easily used in everyday practice to monitor disease activity: the serum CRP levels rapidly increase after a flare-up of the inflammation and decrease following effective immunosuppressive treatment.

In the present study we compared soluble TAA levels with organic involvement (SSc: lung, kidney, Raynaud's phenomenon, joints; SLE: kidney, ling, heart, serositis, nervous system, joints), activity markers (RA: CRP, anti-CCP, DAS28; SSc: CRP; SLE: SLEDAI) and autoantibody production (RA: anti-CCO, RF; SSc: ANA, anti-Scl70, anti-centromer; SLE: ANA, anti-dsDNA, anti-cardiolipin).

2. AIMS

1. To assess the occurrence of secondary malignancies in 516 controlled patients with RA focusing on the lymphoproliferative and solid tumors and to compare the prevalence rates with the general population and the standard morbidity rates (SMR). To examine the effect of immunosuppressive treatment on the development of secondary malignancies in the same patient cohort.

2. To assess the characteristics and frequency of the associated tumors in 218 scleroderma patients based on the abovementioned criteria.

3. To analyze the malignant subpopulation in 860 controlled SLE patients with a special emphasize on the type of cancer, survival rates and correlation to immunosuppressive treatment.

4. To evaluate the serum concentrations of tumor antigens (TAA) also playing a role in the inflammatory process in patients with RA and compare them with healthy controls. To make a comparative analysis between the inflammatory markers of the autoimmune disease (CRP, CCP, DAS28) and the serum TAA levels.

5. To measure the concentrations of the soluble TAA-s in SSc patients and to compare these results with the scleroderma-induced organ-specific manifestations (lung, kidney, joint, Raynaud's phenomenon), the autoantibody levels (ANA, anti-Scl-70, anti-centromer) and the laboratory markers reflecting disease activity (CRP).

6. To assess the concentration of the soluble TAA-s in SLE patients and to compare these results with the characteristic clinical symptoms (kidney, nervous system, heart, lung, serositis), the autoantibody levels (ANA, anti-dsDNA, anti-cardiolipin IgG) and the complex SLEDAI index correlating with disease activity.

3. PATIENTS AND METHODS

3.1. Patients

We analyzed the data of 516 RA and 218 SSc patients from the Department of Rheumatology, Institute for Internal Medicine, University of Debrecen, and 860 SLE patients from the Department of Immunology, 3rd Department of Internal Medicine, University of Debrecen.

Diagnosis of these 3 diseases was made based on the respective diagnostic criteria of the American College of Rheumatology (ACR). We examined the occurrence and basic characteristics of lymphomas and other secondary malignancies in these patients. The levels of circulating TAA-s were assessed in the serum of 75 RA, 92 SSc and 40 SLE patients, and

also in 50 healthy subjects. On the basis of the thorough medical history taken from each patient and the laboratory and imaging tests performed (chest X-ray, abdominal ultrasound or even mammography and endoscopic examinations when necessary) we can say that neither of the patients chosen for TAA evaluation had any previous or concurrent malignant diseases.

3.2. Organ-specific manifestations, laboratory tests and activity scales

Assessment of the clinical manifestations characteristic to the respective disease (kidney, lung, heart, polyarthritis, Raynaud's syndrome, central nervous system, serositis) was carried out with adequate imaging (chest, hand and foot X-rays, chest CT and HR-CT, abdominal US, echocardiography, cranial MRI, capillaroscopy) and laboratory (blood test, urine, respiratory function, biopsy when necessary) examinations.

From the immunological laboratory tests we measured IgM RF and CRP concentrations using quantitative nephelometry. Anti-CCP autoantibodies were detected with a second-generation Immunoscan-RA CCP2 ELISA kit. Assessment of the ANA (on Hep-2 cell), in SSc the anti-topoisomerase I (anti-Scl70) and anti-centromer (ACA) antibodies and in SLE the antibody against the double-stranded DNA (anti-dsDNA) and anti-cardiolipin IgG antibody was performed with standard methods. All these examinations were carried out in the Regional Immunology Laboratory, University of Debrecen.

For the disease activity scales we used the generally accepted DAS28 scoring system in RA and the SLEDAI index in SLE. In SSc no single scale is available that definitely correlates with disease activity so we used CRP level as the inflammatory marker.

3.3. Tumor epidemiology evaluations

We retrospectively analyzed data from 516 RA (1994-2008), 218 SSc (2000-2008) and 860 SLE (1965-2004) patients diagnosed and followed at the outpatient offices of the abovementioned departments using the computer database, patient records and other medical documents. We determined the Standard Incidence Rate (SIR) values in all 3 patient groups but conclusion could be drawn only in the case of SLE.

3.4. Detection of soluble tumor antigens in patients' serum

From the TAA-s CEA, CA19-9, CA15-3, CA125 and CA72-4 were assessed using electrochemiluminescence immunoassay. We determined the percentage of patients with abnormally high TAA levels ('positive') and also evaluated the absolute value of the TAA serum levels, regardless of these concentrations being in the normal or abnormal range. Moreover, TTA levels were compared with the activity markers, organ manifestations and other immunological parameters. During the correlation analysis values under the lower and above the upper cutoff limits were excluded.

3.5. Statistical analysis

For statistical analysis we used the SPSS software package. Normal distribution variables were graphed with mean ±SD values. Data distribution was determined using the Kolmogorov-Smirnov test. Correlation between the TTA serum concentrations and organ-specific manifestations, disease activity scores and other laboratory parameters were calculated with the Mann-Whitney test (level of significance was set to $p<0.05$).

4. RESULTS

4.1. Secondary tumors in rheumatoid arthritis

We analyzed the data of 516 RA patients from the Department of Rheumatology. Those patients were considered "well-controlled" who previously visited our outpatient office at least once a year and also attended follow-ups at least once during 2007-2008.
From the 13 malignancies found, 6 (– almost half the cases –) proved to be bronchial cancer. Another 2 was thyroid cancer (both of follicular type), while a single instance of NHL, breast, gallbladder, colorectal and pancreas cancer was recorded.

4.2. Secondary tumors in systemic sclerosis

We analyzed the data of 218 SSc patients from the Department of Rheumatology. In the evaluated subject group 11 malignant tumors were found in 10 patients. All the SSc patients with malignancies were female and half of the cases (5/10) belonged to the dcSSc clinical subgroup.

4.3. Secondary tumors in systemic lupus erythematosus

860 SLE patients from the Department of Immunology, 3rd Department of Internal Medicine were included in the study. From these patients, cancer developed in 37 cases during the investigation period. The occurrence of malignancies was also evaluated in context of patient age and the results were analyzed broken down to 10-year periods. Patients between 40 and 50 years of age were found to be at the highest risk for cancer development; this age group represented 57% of all the cases. Patients in their 20s and 70s faced almost identical cancer risk. Similarly, 5 cancer cases were registered in both the 30-39 and the 60-69 year patient groups. The order of the incidence of the various cancer types is as follows: ovarian cancer, skin cancer, bladder cancer, lung cancer, hematological cancers, cervix cancer, gastrointestinal cancers and breast cancer.

4.4. Soluble tumor antigens in rheumatoid arthritis

During the assessment of TTA concentrations in RA we found the proportion of RA patients with elevated (above the upper cutoff limit) levels of CA 19-9 (8.1% vs. 0%), CA 125 (10.8% vs. 7.1%) and CA 15-3 (17.6% vs. 14.3%) to be significantly higher compared to the healthy control ($p<0.05$).No major difference was found between RA patients and healthy controls in the CEA (22.2% vs. 21.4%) and CA 72-4 (2.7% vs. 2.1%) concentrations.

When comparing absolute serum concentrations, the levels of CA 19-9 (14.2±1.2 vs. 10.5±1.6 kU/l) and CA125 (23.9±1.8 vs. 16.8±2.2 kU/l) were overall higher in RA than in the controls ($p<0.05$), regardless of the actual values being normal or abnormal in that particular patient. No significant difference was found in the case of CEA (1.8±0.9 vs. 2.6±1.6 μg/l), CA 15-3 (18.6±3.3 vs. 19.2±5.3 kU/l) and CA 72-4 (2.5±1.6 vs. 1.5±1.4 kU/l).

With a pair-wise comparison of the serum concentration of each TAA in RA patients we found significant positive correlation between CA 125 and CA 15-3 ($R=0.377$, $p<0.05$).

Moreover, when comparing activity and prognostic markers with TAA serum levels in the same patient group, a strong correlation was detected between the CEA level and IgM RF concentration (R=0.270, p;0.05). As an internal control, we confirmed that the serum IgM RF level correlates with the anti-CCP (R=0.275, p<0.05) and CRP (R=0.473, p<0.05) production. Neither of the soluble TAA-s was related to serum anti-CCP and CRP levels, nor the DAS28 value reflecting disease activity.

4.5. Soluble tumor antigens in systemic sclerosis

When the percentage of patients with elevated TAA serum concentration was analyzed we found abnormally high CA 19-9 (8.8% vs. 2%), CA 125 (11.0% vs. 6%) and CA 15-3 (28.4% vs. 14%) levels in significantly more SSc patients than in the healthy subjects (p<0.05). No such difference was detected in the case of CEA (20.9% vs. 20%) and CA 72-4 (12.1% vs. 8%).

By comparing the absolute serum levels we observed significantly higher serum concentrations of CEA (6.6±1.7 vs. 1.8±1.4 µg/l) and CA 15-3 (22.9±1.8 vs. 18.6±2.2 kU/l) in the SLE patients, as compared to the controls (p<0.05). There was no significant difference in the CA 125 (21.4±2.7 vs. 23.9±3.2 µg/l), CA 19-9 (14.2±2.1 vs. 14±1.7 kU/l) and CA 72-4 (4.2±3.1 vs. 2.5±3.4 kU/l) levels.

In the SSC patient group the CEA and CA 19-9 serum levels showed a significant positive correlation with each other (R=0.305, p<0.05). The CEA also correlated with the CA 15-3 levels (R%0.259, p<0.05). No such relationship was detected between the serum concentrations of the other TAA-s.

4.6. Soluble tumor antigens in systemic lupus erythematosus

Among the SLE patients with an elevated serum TAA concentration (“TAA positive”) the occurrence of high CEA (32.5% vs. 20.0%), CA 19-9 (7.5% vs. 2.0%), CA 125 (15.0% vs. 6.0%) and CA 72-4 (15.0% vs. 8.0%) levels was significantly more frequent than in the healthy subjects (p<0.05).

Regarding absolute serum concentrations, neither of the TAA-s showed a significant difference (CEA: 3.5±2.8 vs 1.8±1.4 µg/l; CA 125: 19.7±10.9 vs 23.9±3.2 kU/l; CA 19-9: 14.5±3.1 vs 14.0±1.7 kU/l; CA 72-4: 3.0±2.1 vs 2.5±3.4 kU/l; CA 15-3: 17.8±3.8 vs. 18.6±2.2 kU/l) between the patient group and the controls.

Assessing the relationship between the different serum TAA levels in SLE we found a significant correlation between serum CA 19-9 levels and CEA (R=0.580, p=0.009), CA 125 (R=0.500; p=0.029) and CA 15-3 (R=0.589, p=0.008). Soluble CA 125 also correlated with the serum CA 72-4 (R=0.532; p=0.019) and CA 15-3 (R=0.662; p=0.002) levels.
Regarding the association between organ-specific manifestations and soluble markers in SLE, 4 patients had central nervous system involvement. The presence of CNS lupus was in correlation with CA 72-4 levels (Spearman: R=0.624, p=0.004). Serum CA 125 was strongly associated with the SLEDAI disease activity index (R=0.666, p=0.002). No correlation was found between the serum levels of the different TAA-s and the clinical manifestations (skin, heart and lung) or between the serositis and the presence of anti-dsDNA and anti-cardiolipin IgG antibodies.

4.7. Novel results

1. Although the number of secondary malignancies found in the RA patient group was too low for statistical analysis, we reported that more than half of the solid tumors in these patients were bronchial cancer followed by follicular thyroid cancer, breast cancer and hepatobiliary malignancies. Development of NHL was rare. Smoking was considered an important factor of the pathogenesis.

2. The small number of secondary malignancies diagnosed in SSC patients affected only middle-age women and developed mostly in the dcSSc disease subtype. In this group B-cell lymphomas were more dominant than solid tumors from which the slightly elevated incidence of esophageal, cervix and skin cancer should be mentioned.

3. We observed that the cancer morbidity of the SLE patients markedly increased over the years. The most threatened age group is the 40-50 year, and also patients in their 20s and 70s. Solid tumors developed most often in the ovaries and in the skin; from the hematologic cancers NHL had the highest relative risk.

4. In the serum of the RA patients the levels of CA 15-3, CA 19-9 and CA 125 were definitely and significantly higher compared to the healthy controls. No difference was found in the CEA and CA 72-4 levels; regarding the absolute concentration values, only the CA 19-9 was

elevated. Neither of the circulating TAA-s showed any correlation with the serum CRP and anti-CCP values. These latter figures were related to the IgM RF concentration.

5. Regarding the serum level of the soluble markers in patients with scleroderma we found roughly the same patterns and characteristics as in the case of the RA patients, as compared to healthy controls. It must be noted, however, that serum levels showed positive correlation to each other within the population. When comparing the absolute serum levels we found significant difference only in the CEA and CA 15-3. Moreover, in SSc there was a positive correlation between the visceral manifestations, TAA and CRP values and the antibody production.

6. Evaluation of the marker distribution in SLE showed a significant increase in the levels of CEA, CA 19-9, CA 125 and CA 72-4 only, as compared to the control group. However, the absolute concentration values did not reflect such a difference. In "TAA positive" SLE patients the individual TAA levels also correlated to each other. From the organ-specific manifestations (heart, lung, mucosa, etc.) only the nervous system involvement seemed to correlate with serum CA 72-4 and CA 125 levels.

5. DISCUSSION

5.1. Secondary tumors in rheumatoid diseases

5.1.1. Rheumatoid arthritis

Survival rates of the RA patients profoundly improved over the past decades due to the widening of our diagnostic arsenal, including the ability to detect the anti-CCP antibody, better access to US and MRI devices and introduction of the biologic response modifier agents. As a consequence of the better survival rates, chronic co-morbidities and their impact on the overall survival have came into the focus of attention. From the 516 RA patient followed at our department, 13 cases of malignant disease were found (2.5% of all patients). Cancer development followed the onset of RA in 11 patients and preceded it only in the

remaining 2 cases. Although most of the publications found in the literature report the incidence of NHL to increase in this autoimmune disease, in our study the most frequent type of cancer was the bronchial (6 cases, 46%), followed by follicular thyroid tumor (2 cases).We also came across cutaneous NHL, breast-, gallbladder-, colorectal- and pancreas cancer. The average survival was 4.7 years. The small number of malignant cases in our cohort prevents detailed statistical analysis; even though, RA patients seem to face a slightly higher risk of cancer development (SIR: 1.12 compared to the average population data in the Health for All database). Bronchial cancer (10.9, 6 cases) and thyroid cancer (70.7, 2 cases) has the highest SIR. Interestingly, with a single occurrence gallbladder cancer had a SIR of 18.5.

Until recently the role of the B-cells in the pathogenesis of RA and the associated tumors was pretty much underestimated – as opposed to Sjögren's syndrome and SLE –, now it is generally accepted that the chronic B-cell stimulation and increased antibody production is a crucial factor in this disease. It is no wonder that prolonged disease activity and insufficient medical treatment increases the incidence of B-cell lymphoproliferative tumors, while the B-cell inhibitor rituximab may decrease the lymphoma risk. From the solid tumors primarily those become more frequent which develop in the organs affected by the inflammation (e.g. lung).

The most important risk factor is undoubtedly the prolonged activity of the basal disease. Those patients with severe arthritis must face a significantly higher (26-70x) risk of cancer than those in remission. For this reason, despite the controversial approach towards immunosuppressant drugs, it is clear that any articular inflammation must be treated promptly and aggressively. Most of our patients with malignancies belonged to severe RA subtypes: many of them required several DMARD-s and biologic therapy. In these patients we usually detected high IgM RF and anti-CCP serum levels and many of the patients had already undergone one or more articular replacement. Other risk factors include smoking which seems to facilitate the development of both the RA and the secondary malignancies. Almost half of our patients and 83% of those with bronchial cancer smoked. Duration of the disease – particularly if the autoimmune disease was more or less active for years – may also be a pathogenetic risk factor. Our patients had RA for an average of 11 years when the secondary malignancy was diagnosed (with some suffering from the autoimmune disease for more than 20 years). The question of sexual distribution is debated; in our RA population the number of females was 3x higher than of the males, while among the patients with RA and secondary malignancy the proportion of females was even higher (5:1). Moreover, it is well known that the course and prognosis of RA is much less favorable in females which may also indirectly

explain the observed female dominance in the malignant cases. Two patients also had secondary Sjögren's syndrome, which in itself further increases the cancer risk.
Speaking of bronchial cancer, one of the most important risk factors was smoking (5 patients of the 6 smoked) and also the possible role of long-term MTX treatment – which may cause pulmonary fibrosis - must be mentioned Manifest pulmonary fibrosis and consequent bronchial cancer was observed in only 1 case.

mmune suppression is another debated field. The supposedly carcinogenic CPH and AZA are rarely used in the everyday practice these days; in our cohort only 1 patient was administered CPH. In theory, MTX may increase the risk of lymphoma development but on the long term it actually lowers the chance for a secondary malignancy in RA. Large scale metaanalyses from the past years suggest that the TNF-inhibitor biologic drugs may increase the risk of lymphomas. Only 4 patients received biologic treatment in our study which is insufficient number for causality analysis. At our department more than 200 patients with RA received anti-TNF treatment and not a single case of lymphoma development was reported. A comprehensive review from May 2008 concludes TNF inhibitors are unlikely to have direct carcinogenic effect but the cost/benefit ratio has to be considered. When discussing medical treatment and consecutive tumor induction, it must be mentioned that certain drugs require 15-20 years of clinical experience before one could draw conclusions on long-term side-effects.

5.1.2. Systemic sclerosis

Due to the improving survival rates in scleroderma, long-term complications like the occurrence of secondary tumors are becoming more and more problematic. The incidence of renal crisis in SSc dropped significantly; alveolitis, pulmonary fibrosis and pulmonary hypertension is also diagnosed usually at an early stage and several new treatment modalities became easily available. On the contrary, vascular manifestations and secondary malignancies gradually came into the focus of attention.
In this disease the pathogenetic factors leading to chronic inflammatory, autoimmune processes like lasting B-cell stimulation (lymphoma risk) and inflammation (atypia) in the affected organs (in scleroderma: skin, esophagus, lungs) may result in the development of secondary malignancies.
Moreover, certain drugs - primarily the cyclophosphamide - used in the treatment of SSc are supposed to have oncogenic effect.

We recently published a study on the survival data of 336 SSc patients followed in Pécs and Debrecen. Altogether 16 cases of early (diagnosis made within 4 years of the onset of scleroderma) malignancies were registered (4.4%). In the 218 patients with scleroderma followed at our Department only 11 malignant tumors were found (4.6% of all cases); our results match the survival data obtained from the 366 Hungarian patients (4.4%).
As regards the cancer type, we found NHL to be the most frequent malignancy (3 cases, again corresponding to the literature data) followed by breast and lung cancer (2-2 cases), but the occurrence of esophagus, cervix and skin cancer was also observed. Normally the majority of the SSc patients belong to the limited subtype (lcSSc), while exactly the half of our patients 5 cases) with scleroderma and secondary malignancy was of the diffuse (dcSSc) subtype.

5.1.3. Systemic lupus erythematosus

In SLE the early fatal complications are primarily caused by renal insufficiency and various infections, and late complications are characterized by the occurrence of cardio- and cerebrovascular diseases, thromboembolism and malignancies. Cancer morbidity in our patient group was 4.3%, the mortality rate 2%.
Analysis of cancer morbidity in 10-year periods confirmed that the number of patients with malignant disease increased. It is very likely that our data recorded before the 1970 time period cannot be considered reliable. During the past 15 years tumor diagnostic methods improved greatly, probably also contributing to the fact that a higher number of malignancies are diagnosed (and at an earlier stage).
According to our data, most malignancies develop between 40 and 60 years of age. It is important to note that from the 37 patients in 21 (57%) secondary malignancies developed within 10 years after the diagnosis of SLE was made, even if the patients were actually younger than the above mentioned “high-risk” age group.

All patients in our study population received corticosteroid treatment and most of them further immunosuppressant drugs as well. The cumulative impact of these drugs on tumor induction is yet be identified.

5.2. Soluble tumor antigens in rheumatoid diseases

5.2.1. Rheumatoid arthritis

Soluble tumor antigens may be expressed not only on the tumor cells, but also on the surface of inflammatory leukocytes in patients with autoimmune disease and a secondary tumor. We evaluated the levels of CEA, CA 19-9, CA 125, CA 15-3 and CA 72-4 in the serum of 75 RA patients, and compared these data with those of 50 healthy subjects. Each TAA was detectable in both groups. By analyzing the results from the RA group which showed elevated serum concentration – above the upper limit of the normal range – we found that a significantly higher number of RA patient was "TAA positive" for CA 19-9, CA 125 and CA 15-3 than in the control group. Moreover, average serum levels of CA 19-9 and CA 125 were higher in every RA patient, as compared to the controls. There was no such difference in the percentage of "TAA positive" subjects regarding CEA and CA 72-4 levels between the two groups.

In the past very limited data was available on the possible pathogenic, diagnostic and prognostic role of the circulating TAA-s in RA. The CA19-9 antigen was isolated in a whole range of autoimmune diseases including RA. In correlation with other reports, we were able to detect increased expression of CEA on the synovial leukocytes of RA patients. Our results suggest that production of the CA19-9, CA 125 and probably the CA 15-3 is increased in RA compared to the healthy population.

When searching for a prognostic and activity marker among the circulating TAA-s, we also tried to identify any correlation between the different soluble markers and the IgM RF, anti-CCP, CRP and the DAS28 levels. Only the CEA showed a significant association with the RF production. Although serum CEA levels in RA overall were not higher than in the control group, soluble CEA is detectable in the serum of most RA patients and former studies also confirm the synovial expression of the cellular CEA. The possible pathogenic role of the TAAs in RA and other autoimmune diseases is not fully understood. All of the TAA-s assessed in our present study contained such carbohydrate side-branches that take part in cellular adhesion processes during metastasis formation.

As a conclusion, some tumor carbohydrate-like antigens like CA 19-9, CA 125 and CA 15-3 may play a role in cell adhesion justifying their increased production in RA. Similarly, these TAAs- may take part in the adhesive processes following synovial inflammation and production of some of the tumor antigens – e.g. CEA – may be connected to the serum RF

concentration. This means that measurement of the soluble tumor antigens has no prognostic value in RA. TAA-s in SSc and SLE most probably have a similar function as in RA.

5.2.2. Systemic sclerosis

The pathogenic or diagnostic/prognostic value of TAA-s in scleroderma is not known with a only a limited number of literature references. We evaluated the serum concentration of CEA, CA 19-9, CA 125, CA 15-3 and CA 72-4 in our 92 SSc patients and in 50 healthy controls. All the examined TAA-s were detectable in the serum of the SSc patients. In the case of CA19-9, CA125 and CA15-3 "TAA positivity" – TAA concentration above the upper limit of the normal range - was significantly more frequent in the SSc patients than in the controls. Regarding absolute serum concentrations, levels of CEA and CA15-3 were higher in the scleroderma patients than in the controls.

We looked for associations in the SSc population between the TAA-s, organ manifestations and immune-serological alterations and the various marker levels. Serum CEA showed significant correlation to the CA19-9 and CA15-3 levels. The levels of solubile CEA, CA 15-3 and CA 19-9 correlated with the renal insufficiency in SSc, the CA15-3 also with occurrence of polyarthralgia/arthritis, ANA positivity and CRP level. The CRP, used as a marker for disease activity, also showed some correlation to the CA15-3.

5.2.3. Systemic lupus erythematosus

We evaluated the TAA levels in 40 SLE patients and compared them to 50 healthy controls. In the case of CEA, CA 19-9, CA 125 and CA 72-4 "TAA positivity" was significantly more frequent in patients with SLE than in the controls. By measuring absolute serum concentrations we found no difference in either of the markers between the two groups.

Regarding the association between the different TAA-s, and between those antigens and organ-specific manifestations, soluble CA 19-9 showed significant correlation with the level of CEA, CA 125 and CA 15-3 tumor markers. Moreover, association was found between the CA 72-4 production and the nervous system involvement (neuron-lupus). The SLEDAI is a widely used complex lupus activity index. In our study the soluble CA125 showed correlation with the SLEDAI score.

6. SUMMARY

Due to the early diagnosis, modern therapeutic modalities and close follow-up of patients in rheumatology centers, there has been a substantial increase in the survival of rheumatoid arthritis (RA), lupus (SLE) and scleroderma (SSc) patients.

As a consequence, long-term organ damage and chronic comorbidities, primarily vascular disorders and secondary malignancies have become major issues in relation to these diseases. There is increased cancer morbidity and mortality in autoimmune-rheumatic diseases. Furthermore, immunosuppressive therapies administered to RA, SLE as well as SSc patients may exert bimodal action on the incidence of secondary tumors. Yet, sustained inflammation is the primary risk factor in the development of malignancies among these conditions. Therefore, it is very important to diagnose and treat these patients early and tumor screening may be crucial in patients at high risk.

Tumor-associated antigens (TAA) may be involved in the pathogenesis and/or laboratory diagnostics of autoimmune-inflammatory diseases. By their sialylated carbohydrate motifs, they are involved in cell adhesion, thus most TAA-s are expressed on the surface of inflammatory leukocytes, as well as tumor cells. TAA-s exert an important role in inflammation and metastasis formation. Shedding from the cell surface, they become soluble and detectable in the patients' sera. Results of this study indicate that the production of some TAA-s, such as CEA, CA 19-9, CA 125, CA 15-3 and CA 72-4, may be increased in RA, SLE and SSc in comparison to control subjects. In addition, serum concentrations of some TAA-s are correlated with indicators of disease activity, as well as some organ manifestations. Thus the detection of solubile antigens may have pathogenic, clinical and prognostic relevances in autoimmune rheumatic diseases.

Assessment of the clinical aspects of secondary malignancies and soluble TAA-s may help us to understand similar mechanisms of autoimmunity and tumorgenesis. These data also emphasize the important role of early diagnosis, screening, agressive treatment and close follow-up of autoimmune-rheumatic diseases.