Autoantibodies against lamin C, NA14 and CK15 in primary vasculitides or autoimmune diseases with secondary vasculitis

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ABSTRACT

Objective. *Autoantibodies may play a role in the pathogenesis of primary vasculitides (PVs) like giant cell arteritis (GCA). We recently identified 3 cytoskeletal proteins (lamin C [laC], nuclear autoantigen of 14kD [NA14] and cytokeratin 15 [CK15]) as autoantigens in GCA and postulated a frequent autoantibody response against these antigens in PVs.*

Methods. *To test this hypothesis we studied a cohort of patients with PVs (n=61) and healthy individuals (n=27) for the presence of these autoantibodies using a recombinant cDNA expression library. To define their specifity for PV, we also examined patients with other autoimmune diseases such as rheumatoid arthritis (RA) and connective tissue diseases (CTD).*

Results. *We found no statistically significant differences in autoantibody responses between patients with PV and healthy controls, although there was a trend for an association between PVs and the occurrence of antibodies against laC and CK15. However, in patients with RA (n=33) or Sjögren's syndrome (SS, n=11) with vasculitides we observed more frequently autoantibodies against NA14, laC and CK15 compared to healthy controls. In patients with systemic lupus erythematosus (SLE, n=23) autoantibodies against laC were more frequent. The presence of autoantibodies in RA, SS and SLE was associated with systemic secondary vasculitis (SSV). In RA, laC- and NA14-seropositive patients were in a more advanced disease stage than seronegative patients with more frequent extraarticular manifestations (p=0.004). In SLE laC-autoantibody-positive patients had a higher SLE activity index (p<0.001).*

Conclusion. *Serum autoantibodies against laC and NA14 are frequent in patients with RA and CTD and are associated with extensive disease and SSV. The potential pathogenic and prognostic role of these antibodies should be further investigated.*

Introduction

Systemic vasculitides represent a heterogeneous group of diseases characterised by the presence of inflammatory lesions within the vessel wall. In general, affected vessels vary in size, type, and location in association with the specific vasculitic disorder (1). Vasculitis may occur as a primary process or may be secondary to another underlying disease. Primary systemic vasculitides are classified according to the size of the injured vessels in line with the existing classification schemes (2-4). Secondary vasculitides are observed *e.g.* in a subset of patients with rheumatoid arthritis, systemic lupus erythematosus, and other connective tissue disorders who develop a vasculitis associated with the systemic disease. In agreement with the current state of knowledge different pathogenic mechanisms - in many cases on the basis of a multifactorial process that includes genetic predisposition, environmental adjuvant factors and immune dysregulation – underlie the respective vasculitic disease. In primary vasculitides *e.g.* proinflammatory cytokine production, macrophages and T-cell activation dominate in large-vessel vasculitides, such as giant cell arteritis (GCA) (5). Deposition of soluble immune complexes responsible for complement classical pathway activation and neutrophil recruitment prevail in medium-sized vessel vasculitides, such as polyarteritis nodosa (PAN) related to hepatitis B virus (HBV) infection (6); and neutrophil activation by anti-neutrophil cytoplasm antibodies (ANCA) play a major role in ANCA-positive small-vessel vasculitides (APVA), such as granulomatosis with polyangiitis (GPA, Wegener's granulo-

matosis) and microscopic polyangiitis (MPA) (7). However, the pathogenesis of primary and secondary vasculitides remains incompletely elucidated and may require additional factors. In this regard little is known about the role of B cells and autoantibodies, especially in ANCA-negative primary vasculitides like PAN, Takayasu`s arteritis (TA) or GCA. The same holds true for secondary vasculitides in rheumatoid arthritis (RA) and connective tissue diseases like systemic lupus erythematosus (SLE) (8). On the other hand, there are some recent studies which give hints to the contribution of self-reactive B cells in ANCA-negative vasculitic diseases. For example, anti-endothelial cell antibodies (AECAs) have been demonstrated in a spectrum of systemic inflammatory diseases, including primary and/or secondary systemic vasculitis. These antibodies may cause endothelial cell activation (9) and the induction of antibody-dependent, cell-mediated cytotoxicity and apoptosis (10). Anti-progranulin antibodies were detected in GCA, TA, PAN or GPA and their proinflammatory effects might be explained by blocking the anti-inflammatory effects of progranulin, thus contributing to the pathogenesis of the respective vasculitis (11). Unfortunately, the microbial or self antigens supposed to trigger an immune-mediated reaction in ANCA-negative primary and secondary vasculitides, including autoreactive B cells and autoantibodies, are largely unknown, especially in large-vessel vasculitides like GCA (5, 12). So far, these target antigens have been identified by chance rather than by systematic search. Therefore, in an unbiased approach we recently explored the antibody repertoire of patients with GCA as a representative disease for ANCA-negative vasculitides for the presence of autoantibodies against self antigens. To this end, we screened sera from 3 patients with GCA for autoantigen-IgG antibodies reactive with a cDNA library derived from normal human testis using SEREX, the serological identification of antigens by recombinant cDNA expression cloning (13). We identified 3 different target antigens, which reacted preferentially with sera from patients with GCA in comparison with healthy individuals. These antigens include the nuclear autoantigen of 14 kD (NA14, also called Sjögren's syndrome nuclear autoantigen 1, SSNA1); lamin C which belongs to the nuclear lamin-forming protein family of lamins; and cytokeratin 15 (CK15), which is a type I keratin responsible for the structural integrity of epithelial cells.

On the basis of these findings we now investigated the frequency of IgG antibody responses against these target antigens in a clinically well defined cohort of patients in order to define their specificity for systemic primary (GCA, TA, APVA) and secondary vasculitides in RA, SS and SLE. The results should help to understand the role of an antibody response for the pathogenesis of the respective autoimmune disease and their activity.

Material and methods

Patients and sera

After informed consent, serum samples from 128 patients with GCA, TA, APVA and other autoimmune disease and from patients with cancer and sepsis (n=29) were obtained at the Dept. of Internal Medicine I, Saarland University Medical School, Homburg, Germany. Healthy persons were volunteers from Germany, without evidence of infection, autoimmune disease, cancer or cardiovascular disease. All patients and healthy controls were Caucasians. The study had been approved of by the local ethical review board (Ethikkommission der Ärztekammer des Saarlandes) and the Declaration of Helsinki principles were followed. The diagnosis of GCA was based on characteristic clinical findings and was confirmed by temporal artery biopsy consistent with the American College of Rheumatology (ACR) 1990 criteria of giant cell arteritis (14). Patients with TA, granulomatosis with polyangiitis (Wegener's; GPA) and microscopic polyangiitis (MPA) fulfilled the ACR 1990 criteria for TA, GPA and MPA (15, 16). All patients with RA fulfilled four or more of the 1987 ACR and the revised ACR/EULAR criteria from 2010 (17, 18). The extraarticular manifestations studied were clinically diagnosed (*e.g.* pericarditis) with other causes unlikely or excluded. Major cutaneous vasculitis was defined as biopsy-proven necrotising or leucocytoclastic vasculitis. All patients with SS fulfilled 4 or more of the diagnostic criteria for SS proposed by the European Community Study Group in 1993 (19). Diagnostic tests for SS (*e.g.* rose Bengal staining, Schirmer test) were performed according to these recommendations. All patients underwent minor salivary gland biopsy, which was found to be positive in 90%, using Chisholm and Masson criteria (20). The patients with SLE met at least four of the ARA criteria for the classification of SLE (21). The SLE disease activity index (SLEDAI) was calculated from signs and symptoms recorded at the time of sampling sera (22). The cohorts of RA, SS, and SLE were stratified into subgroups of systemic manifestations such as presence or absence of major organ involvement based on clinical observation; in addition, these patients were stratified into patients with or without histologically proven vasculitis by biopsy. Laboratory assessments for all patients with autoimmune disease and healthy controls included complete blood cell count, erythrocyte sedimentation rate (ESR), and serum levels of C-reactive protein (CRP), IgG, IgA, IgM, C3, C4, CH50, rheumatoid factor (RF), anti-nuclear antibody (ANA), and anti-DNA antibody. The cohorts of RA, SS, and SLE were stratified into subgroups of systemic manifestations such as major organ involvement based on clinical observation; furthermore, the patients were further stratified into patients with histologically proven vasculitis by biopsy.

Sera and tissue

Sera were obtained during routine diagnostic or therapeutic procedures. Sera were stored at -80°C until use. Normal testis tissue was obtained from an orchidectomy performed as a hormone-ablative measure in a patient with metastatic prostate carcinoma.

Construction of the cDNA expression library

Recombinant DNA work was done with the official permission and according to the rules of the state government of

Saarland. Fresh testis tissue was used to establish a cDNA library as described by us for malignant tissues (13).

Phage assay for detection of target antigens in autologous sera

We screened 1:250 diluted autologous sera from three patients with untreated GCA/PMR, which had been preabsorbed with transformed *Escherichia coli,* for IgG reactivity against transfectants derived from the above described testis cDNA expression library (13). Briefly, *E. coli* transfected with recombinant, AZAPII phages were plated onto Luria-Bertani agar plates. Expression of recombinant proteins was induced with isopropyl P-D-thiogalactoside. Plates were incubated at 37°C until plaques were visible and then blotted onto nitrocellulose membranes. The membranes were blocked with 5% (wt/vol) low-fat milk in Tris-buffered saline and incubated with the respective dilution of the patient's serum, which had been preabsorbed with transfected *E. coli*. Serum antibodies binding to recombinant proteins expressed in lytic plaques were detected by incubation with an alkaline phosphatase-conjugated goat anti-human IgG and visualisation by staining with 5-bromo- 4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Suppl. Fig. 1a). The sequence of the coding DNA (cDNA) for these recombinant proteins or possible target antigens, respectively, was analysed as described below.

Sequence analysis of target antigens

Positive clones coding for possible target antigens were subcloned to monoclonality and subjected to *in vivo* excision of pBK-CMV phagemids. The nucleotide sequence of cDNA inserts was determined and sequence alignments were done as previously described (23).

Phage assay for the detection of IgG antibodies in allogenic sera

In order to define the role of possible target antigens in patients with vasculitis we determined the frequency of IgG autoantibodies in patients with primary vasculitis and autoimmune disease associated with vasculitis. For this purpose, phages containing the target antigen sequence (*e.g.* lamin C) were mixed with a non-reactive phage of the same cDNA library containing tripeptidyl-peptidase-II (TTP2) (24) as internal negative control at a ratio of approx. 1:10 and used to transfect bacteria. Escherichia coli-absorbed sera (1:100, 1:250 or 1:1000 diluted) from patients, control patients with cancer and sepsis as well as healthy controls were tested for the presence of IgG antibodies reactive with the respective clone using the above described phage assay. In this way we obtained assay filters (nitrocellulose membranes) with stained reactive and unstained non-reactive plaques (Suppl. Fig. 1a). Scanned images of the assay filters were analysed with Image Quant (Molecular Dynamics, Sunnyvale, CA, USA) for the assessment of staining intensity. The staining intensity was determined by the measurement of density of the reactive plaques relative to the non-reactive plaques (Suppl. Fig. 1b). A positive signal was defined as a staining intensity of the reactive plaques that is at least 2.5 or more times the staining intensity of non-reactive plaques.

Statistical analysis

Comparison between patients and healthy donors were done in cross-tables and were tested by Chi-square or Fishers's exact test when appropriate. Correlation between IgG responses and activity of diseases was analysed by Mann-Whitney U-test (MWU). Standard error values are presented as error bars in graphs. In order to interpret the significance of differences observed, *p*-values <0.05 were considered as significant.

Table I. Patients' characteristics.

Diagnosis	Number	Median Age	Sex (female) n. (%)	Systemic disease* (%)	Disease activity** (median)	Systemic vasculitis n. (%)
Giant cell arteritis	43	69	28 (65)	N/A#	N/A#	43 (100)
Takayasu arteritis	8	49	8 (100)	N/A#	N/A#	8 (100)
ANCA+vasculitis	10	59	5 (50)	N/A#	8	10 (100)
Rheumatoid arthritis	33	52	23 (70)	10 (30)	N/A#	3 (9)
Sjögren's syndrome	11	42	9 (82)	7 (64)	N/A#	2 (18)
Systemic lupus eryth.	23	38	21 (91)	N/A#	9	3 (13)
All autoimmune disease	128	59	94 (73)	N/A#	N/A#	69 (54)
Healthy persons	27	60	22 (82)	N/A#	N/A#	0 (0)
Cancer patients	25	59	14 (56)	N/A#	N/A#	0 (0)

*Extraglandular or extraarticular disease; N/A: not applicable; **median points of Birmingham vasculits activity score and SLEDAI, #no clear definition of systemic disease or disease activity available.

Results

Patients' characteristics

We tested the IgG response against lamin C, NA15 and CK15 in 61 patients with primary vasculitis: 43 patients with GCA, 8 patients with TA and 10 patients with APVA. In order to study whether a response against these antigens is also found in other autoimmune diseases, we investigated the frequency of IgG autoantibodies in 67 patients with these diseases: 33 patients with RA, 11 patients with SS, and 23 patients with SLE. Healthy individuals (n=27) and patients with cancer (n=25) or sepsis (n=4) served as controls. Median age and sex of the patients with primary vasculitis and other autoimmune disease are shown in Table I for the respective type of disease. The proportion of patients with systemic disease defined as extraarticular or extraglandular disease and the proportion of patients with systemic vasculitis are also presented in Table I. Where applicable disease activity index is shown in Table I. The healthy control group and patients with cancer or sepsis were matched with the patients with primary vasculitis and autoimmune diseases for age and sex. Age with a cut-off at 60 years did not influence the occurrence of antibodies against lamin C, CK15 and NA 14. For more detailed information about clinical characteristics of the patients see supplemental results.

IgG antibodies in patients with primary vasculitis and other autoimmune diseases

In 61 patients with primary vasculitis there was a trend for a higher fre-

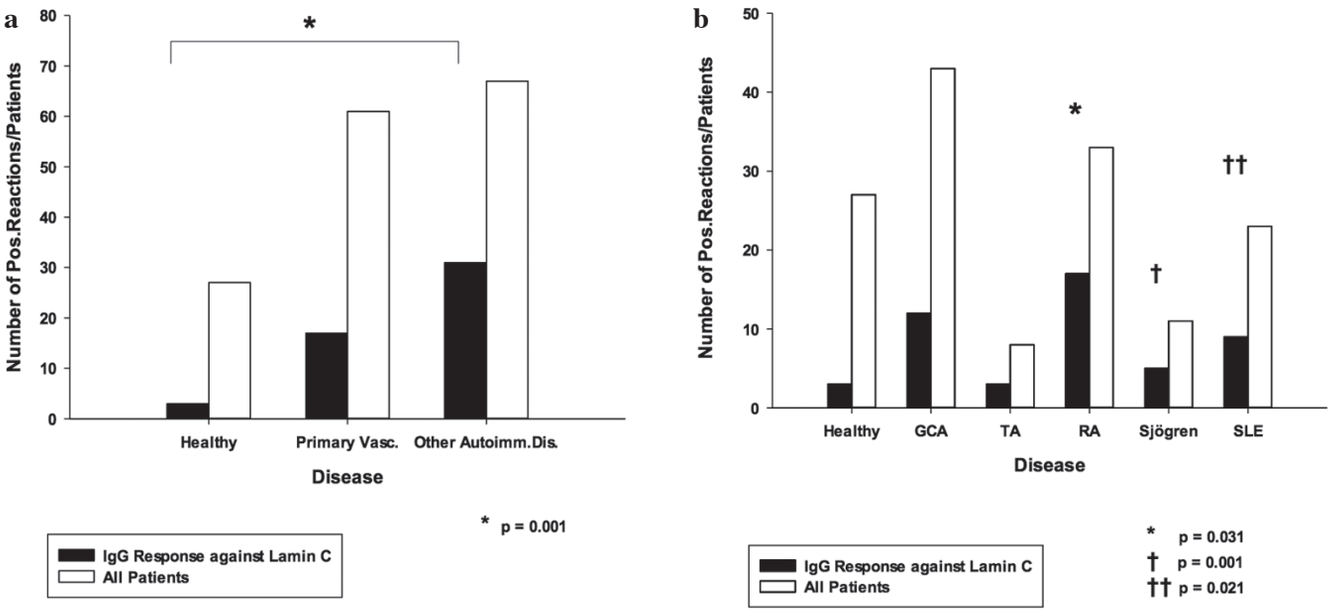

Fig. 1. Autoantibodies against Lamin C

a. Primary *vs*. Secondary vasculitis. Healthy persons (n=27), patients with primary vasculitis (n=61) and patients with secondary vasculitis in autoimmune disease (n=67) were tested for IgG antibodies against laC, which was more frequent in patients with autoimmune disease with secondary vasculitis (black bars, 46%) than in patients with primary vasculitis (28%) in comparison to healthy individuals (11%). **p*=0.001, Chi-square test.

b. Vasculitis subgroups. IgG response against laC in patients with rheumatoid arthritis (RA) (52%), Sjögren's (46%, n=11) and SLE (39%), but not in pateients with giant cell arteritis (28%) or Takayasu arteritis (37%, n=8), was significantly higher than in healthy persons (11%). **p*=0.03, †*p*=0.001, ††*p*=0.02 Chi-square test.

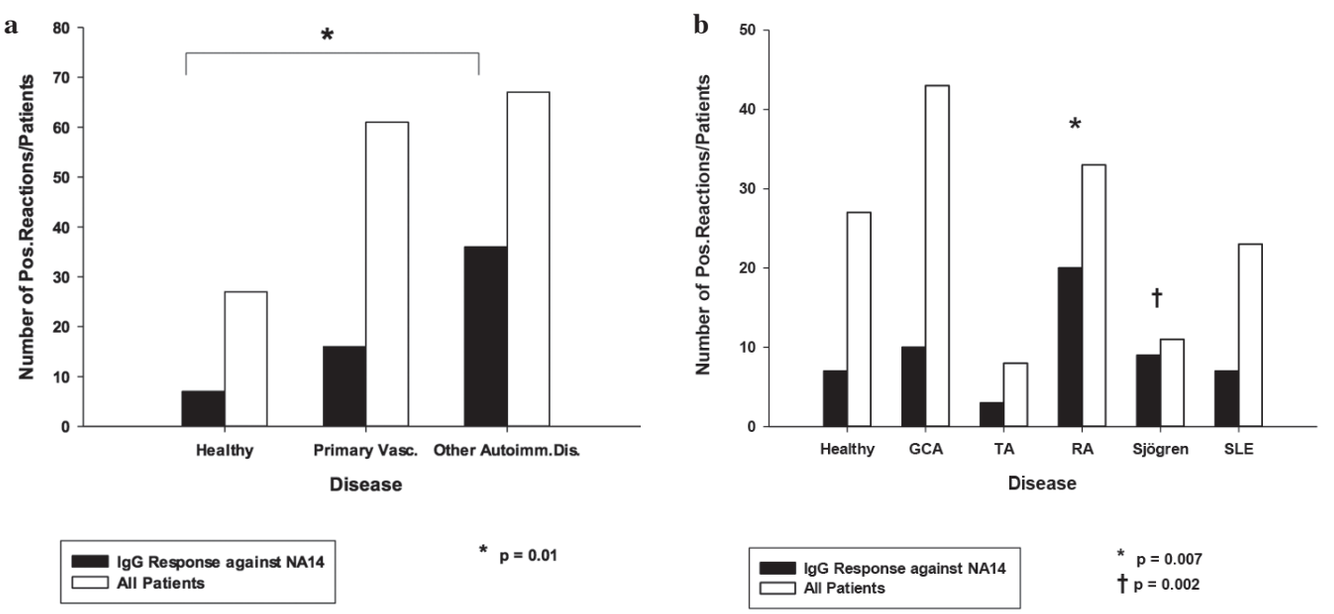

Fig. 2. Autoantibodies against NA14.

a. Primary *vs*. Secondary vasculitis. Healthy persons (n=27), patients with primary vasculitis (n=61) and patients with secondary vasculitis in autoimmune disease (n=67) were tested for IgG antibodies against NA14, which was more frequent in patients with autoimmune disease with secondary vasculitis (black bars, 54%) than in patients with primary vasculitis (26%) in comparison to healthy individuals (26%). **p*=0.01 Chi-square test.

b. Vasculitis subgroups. IgG response against NA14 in patients with RA (61%) and SS (82%) was significantly higher than in healthy persons (26%). **p*=0.007, †*p*=0.002, Chi-square test.

quency of IgG responses against laC versus healthy individuals (*p*=0.08), but the difference was not statistically significant. In contrast, we found that an IgG response against laC was more frequent (46%) in 67 patients with other autoimmune diseases (RA, SS, SLE) in comparison to healthy individuals (11%, *p*=0.001, Chi-square test, Fig. 1a). This was also true for the IgG response against NA14 (54% *vs*. 26%, *p*=0.01, Fig. 2a) and CK 15 (33% *vs*. 7%, *p*=0.01, Fig. 3a). In a more detailed analysis, we showed that the IgG response against laC in patients with

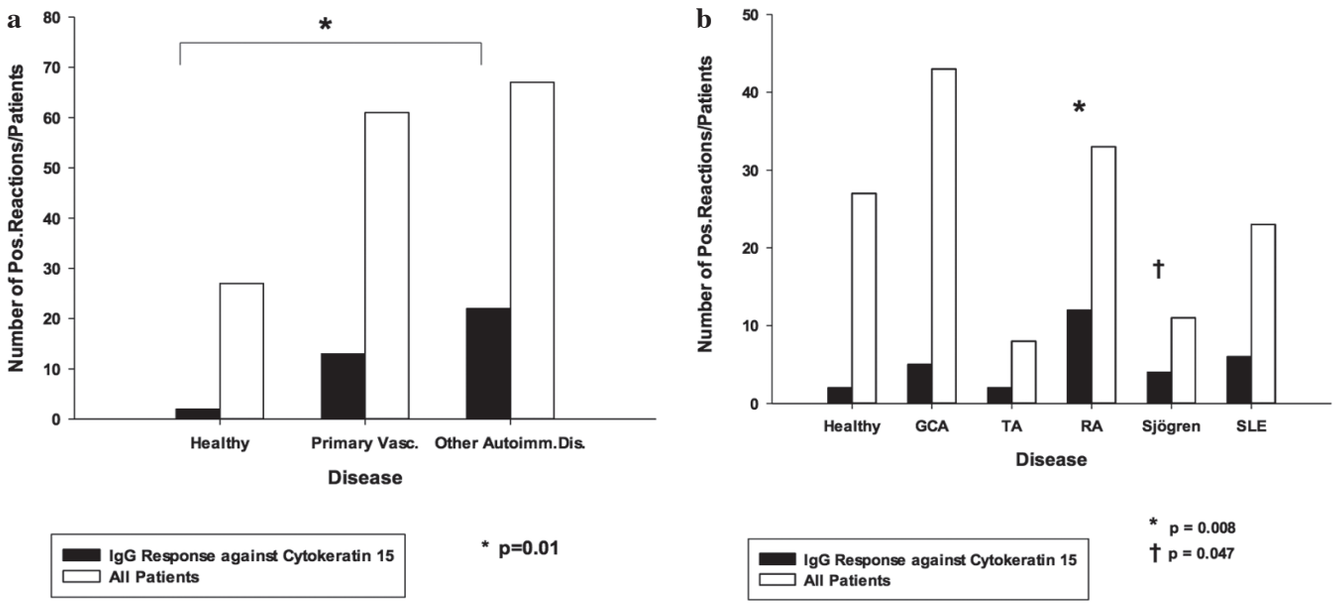

Fig. 3. Autoantibodies against CK15.
a. Primary *vs*. Secondary vasculitis. Healthy persons (n=27), patients with primary vasculitis (n=61) and patients with secondary vasculitis in autoimmune disease (n=67) were tested for IgG antibodies against CK15, which was more frequent in patients with autoimmune disease with secondary vasculitis (black bars, 33%) than in patients with primary vasculitis in comparison to healthy individuals (7%). **p*=0.01 Chi-square test.
b. Vasculitis subgroups. IgG response against CK15 in patients with rheumatoid arthritis (RA) (36%) and Sjögren's (36%) was significantly higher than in healthy persons (7%). **p*=0.008, †*p*=0.05, Chi-square test.

RA (52%), SS (46%) and SLE (39%), but not with GCA, TA or APCA, was significantly higher than in healthy persons (11%, Fig. 1b). This also applied to the IgG response against CK15 in patients with RA (36%) and SS (36%, Fig. 3b) as well as to the IgG response against NA14 in patients suffering from RA (61%) and SS (82%, Fig. 2b) with the latter IgG response demonstrating the highest frequency. In order to prove the reproducibility of the experimental results and to get information about the strength of the response we tested the IgG responses at 3 dilutions (1:100, 1:250 and 1:1000) demonstrating that – despite lower absolute numbers of IgG responses at higher dilutions - the majority of IgG responses at 1:100 are also detected at a dilution of 1:250 or 1:1000 (as an example compare IgG responses against LaC at 1:100 and 1:1000 in Fig. 1b and 4c). Even though in patients with primary vasculitis the frequency of IgG responses against laC, CK15 and NA14 at a dilution of 1:100 was not low, the difference between patients with primary vasculitis and healthy persons was not statistically significant (p=0,08 and 0,11, Fig. 1a and 3a, respectively) or not present (NA14: 26.2 *vs*. 25.9%, p=0,97, Fig. 2a). Rather, a statistically significantly higher frequency was shown in patients with autoimmune diseases in which secondary vasculitis is a possible manifestation of the disease (see above). It is important to note that only a small proportion of these patients had a histologically proven vasculitis (8/67 or 12%). Interestingly, all these patients demonstrated an IgG response against lamin C or NA14 (8/8 each), but only 39% or 48%, respectively, of the patients without a proven vasculitis (Fig. 6a, p=0.001 or Fig. 6b, 0.006, Fisher's exact test). Very similar results were observed for IgG responses against CK15 (Fig 6c, p=0,001, Fisher's exact test). In addition, we observed a non-significantly elevated frequency of IgG responses against Lamin C, CK15 and NA14 in patients with cancer. In patients with sepsis (n=4) we did not find IgG responses against the above mentioned target antigens.

Semiquantitative analysis of IgG antibodies in patients with primary vasculitis and other autoimmune diseases

The above reported results reflect the frequency of the antibody response against laC, NA14 and CK15 for a certain – relatively low – antibody concentration or titer, respectively (serum dilution 1:100). For this concentration we found no statistically significant differences between the frequency of antibodies against cytoskeletal antigens in patients with primary vasculitides and healthy persons, although there was a trend for an association between primary vasculitides and the occurrence of antibodies against laC and CK15 (p=0.084 and 0.11, respectively). However it may be possible to verify differences between patients and healthy individuals, if these both groups are examined with respect to high-titered antibody responses. For this reason we assessed the antibody responses in a semiquantitative approach at a serum dilution of 1:250 and 1:1000. For high-titered antibody responses against NA14, we found – concordant with the observation in low-titered antibody responses - that in patients with primary vasculitis the frequency of antibody responses was not higher than in healthy individuals (data not shown). For high titered antibodies against laC we confirmed the trend for an association with primary

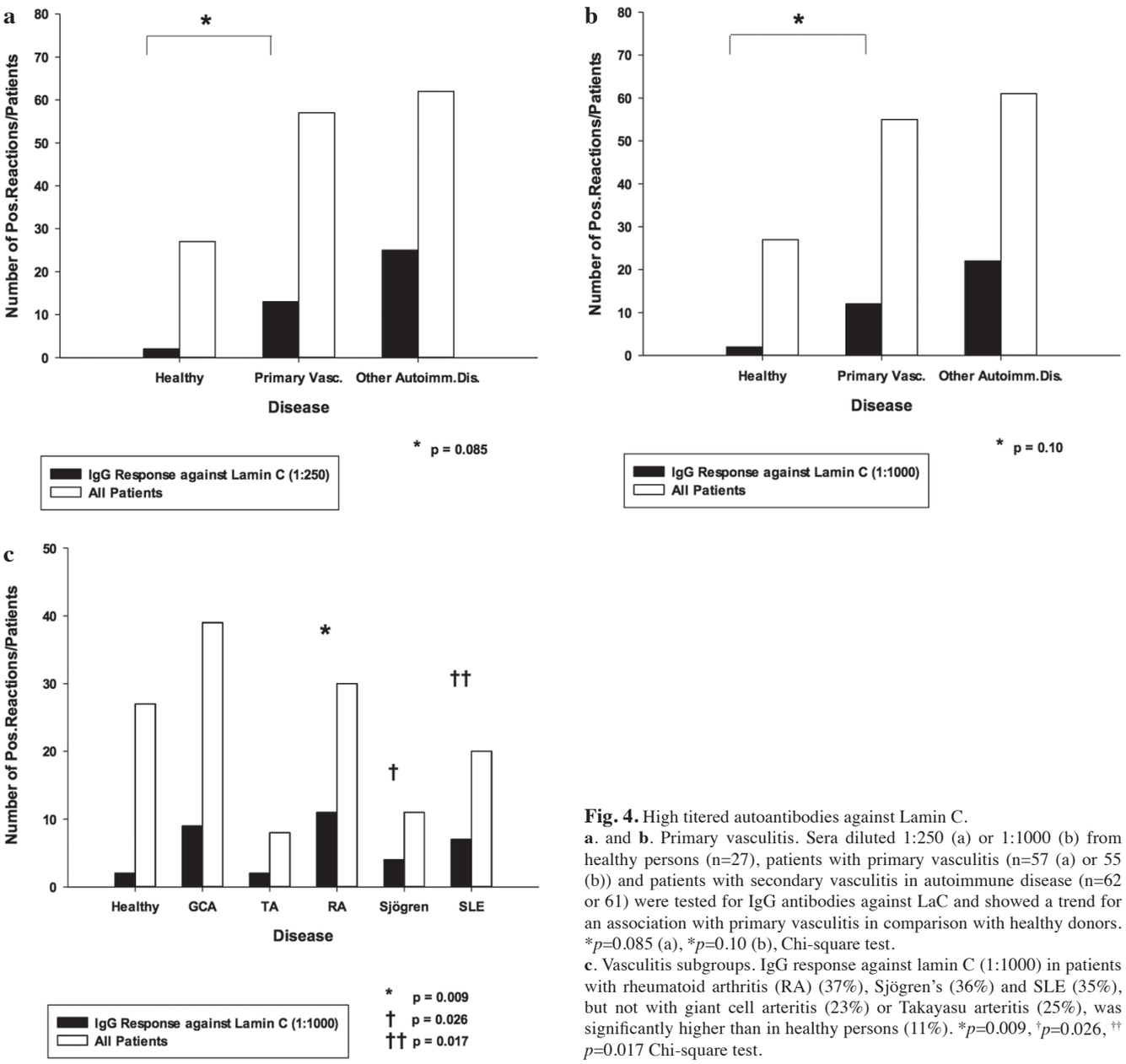

Fig. 4. High titered autoantibodies against Lamin C.
a. and **b**. Primary vasculitis. Sera diluted 1:250 (a) or 1:1000 (b) from healthy persons (n=27), patients with primary vasculitis (n=57 (a) or 55 (b)) and patients with secondary vasculitis in autoimmune disease (n=62 or 61) were tested for IgG antibodies against LaC and showed a trend for an association with primary vasculitis in comparison with healthy donors. **p*=0.085 (a), **p*=0.10 (b), Chi-square test.
c. Vasculitis subgroups. IgG response against lamin C (1:1000) in patients with rheumatoid arthritis (RA) (37%), Sjögren's (36%) and SLE (35%), but not with giant cell arteritis (23%) or Takayasu arteritis (25%), was significantly higher than in healthy persons (11%). **p*=0.009, †*p*=0.026, †† *p*=0.017 Chi-square test.

vasculitis (Fig. 4a and b), which was already shown for low-titered antibodies. The same was true for high-titered antibodies against CK15 (Fig. 5a and b), for which we detected more frequent antibody responses in patients with primary vasculitis *versus* healthy persons with borderline statistical significance (*p*=0.056). For patients with secondary vasculitis we confirmed that the elevated frequency of autoantibodies against laC, NA14 and CK15 in comparison to healthy donors, which was shown at serum dilution 1:100, is also present in high-titered antibody responses. For example, the frequency of antibody responses against laC at serum dilution 1:1000 in patients with RA, SS and SLE was higher than in healthy persons (as shown in Fig. 4c, data for NA14 and CK15 not shown).

IgG antibodies and extension /activity of disease

Some known autoantibodies in autoimmune disease are associated with the extent and activity of the respective autoimmune disease. Therefore, we investigated whether there is an association between IgG responses against laC, NA 14 and CK15 and the extent or acitivity of primary vasculitis or other autoimmune disease. In RA (n=33) the occurrence of IgG antibodies against laC (dilution 1:100) was associated with the number of swollen joints (*p*=0.001, Mann-Whitney U-test): in anti-laC negative patients the mean number of swollen joints was 2.5, whereas there were 4 swollen joints in laC-seropositive patients. There was no association between rheumatoid factor and IgG antibodies against laC, CK15 and NA14. LaC- and NA14-seropositive patients were usually in a more advanced disease stage than laC- and NA14-seronegative patients, as shown by more frequent extraarticular manifestations

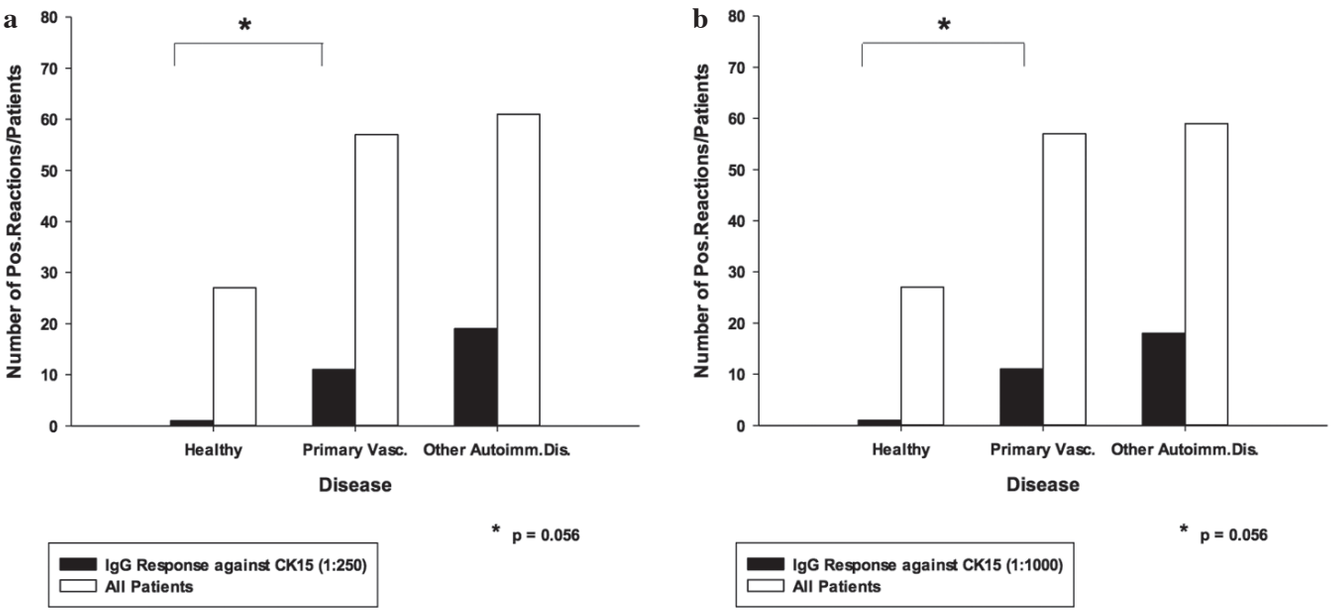

Fig. 5. High titered autoantibodies against CK15 – Primary vasculitis. Sera diluted 1:250 (a) or 1:1000 (b) from healthy persons (n=27), patients with primary vasculitis (n=57) and patients with secondary vasculitis in autoimmune disease (n=61 (a) or 59 (b)) were tested for IgG antibodies against CK15. An IgG response against CK15 was – with borderline statistical significance - more frequent in patients with primary vasculitis in comparison to healthy individuals. *p=0.056, Chi-square test in both (a) and (b).

of the disease (53% and 45%) compared to seronegative cases (6% and 8%, p=0,004 and p=0,02, Chi-square test, Fig. 7a and b). In SS, laC antibodies were detected more often in patients with extraglandular disease (5/5) than without (2/6, p=0.045 Fisher's exact test). There was no association between ANA or anti-Ro/anti-La-antibodies and IgG antibodies against laC, CK15 and NA14. In SLE (n=23) the occurrence of IgG antibodies against laC was associated with the SLE activity index (SLEDAI, p<0.001, Mann-Whitney U-test): in laC-seronegative patients the median SLEDAI was 5, whereas SLEDAI was 13 in lamin C-seropositive patients (Fig. 8). There was no association between ANA or anti-DNS antibodies with IgG antibodies against lamin C, CK15 and NA14.

Discussion

We report here the autoantibody response against the antigens lamin C, NA14 and CK15 in patients suffering from primary vasculitis (GCA, TA, APVA) or other autoimmune disease (RA, SS, SLE). In RA and connective tissue diseases (SS, SLE) we demonstrated a significantly more frequent antibody response against at least one of these autoantigens in comparison to healthy individuals, with a response rate ranging between 36 and 82%. In contrast, there was no statistically significant difference between the antibody response rate against these 3 autoantigens in patients with primary vasculitis compared to healthy individuals, although there was a trend for an association between primary vasculitides and the occurrence of antibodies against LaC and CK15. This was a slightly surprising finding, because earlier investigations in a smaller group of patients suggested that autoantibodies against these antigens are present at higher frequencies in patients with primary vasculitis like GCA (23). There are different possible explanations for this finding: 1. pathogenic mechanisms: it may be that in primary vasculitis like GCA and TA autoantibodies are present at low frequencies and T-cells dominate the pathogenic immune response (25-28) - despite there are some hints that B-cell responses and autoantibodies play a role in pathogenesis (29-31). 2. Statistical reasons: we investigated a limited number of patients with primary vasculitides. The statistical power of this investigation may be too low to detect the difference. 3. Methodological reasons: we cannot exclude that with the used experimental technique (phage assay (13)) for autoantibody screening some autoantibodies are not detected, because they escape from analysis. However, this problem is also present in other experimental techniques like protein macroarrays (11) or 2D-electrophoresis and immunoblotting (32) and is caused *e.g.* by antigens which undergo post-translational modification like glycosylation (not present in prokaryotic expression systems) or membrane proteins (because of their hydrophobicity their analysis is hampered in most two-dimensional gel electrophoresis techniques).

On the other hand, we showed a clear difference in autoantibody responses in RA and connective tissue disease versus healthy persons. The analysis of patients with RA, SS and SLE with histologically proven secondary vasculitis (8 of 67) demonstrated in all cases (n=8) an antibody response against lamin C, NA14 and CK15, but only in 40% of the other cases without vasculitis. This does not necessarily mean that antibody response against lamin C, NA 14 and CK15 are specific for secondary vasculitis, because in all these eight patients other organs (e.g. joints, skin, glandula parotis) were also affected by the autoimmune process. In summary, antibody responses against the above-

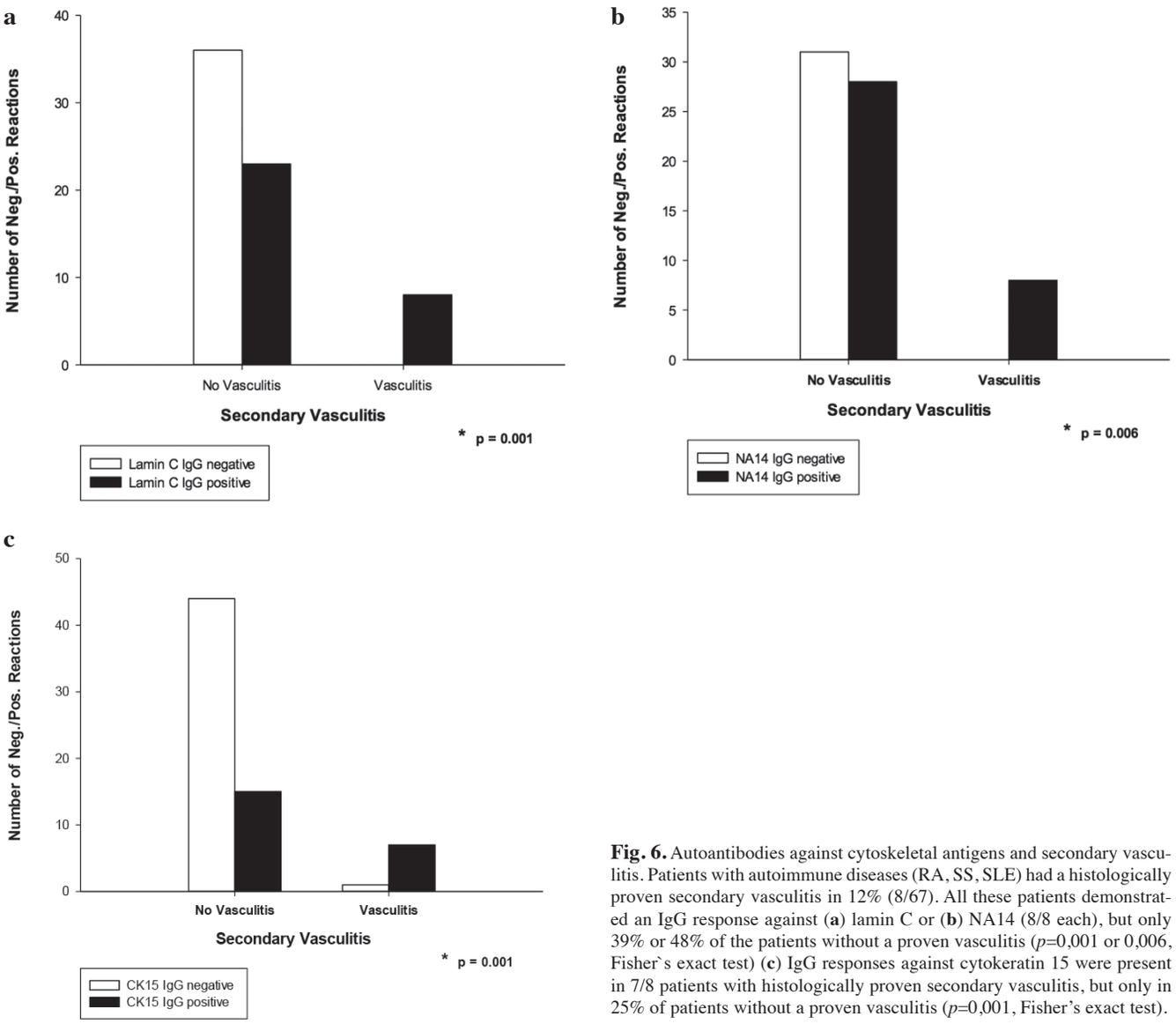

Fig. 6. Autoantibodies against cytoskeletal antigens and secondary vasculitis. Patients with autoimmune diseases (RA, SS, SLE) had a histologically proven secondary vasculitis in 12% (8/67). All these patients demonstrated an IgG response against (**a**) lamin C or (**b**) NA14 (8/8 each), but only 39% or 48% of the patients without a proven vasculitis (p=0,001 or 0,006, Fisher`s exact test) (**c**) IgG responses against cytokeratin 15 were present in 7/8 patients with histologically proven secondary vasculitis, but only in 25% of patients without a proven vasculitis (p=0,001, Fisher's exact test).

mentioned autoantigens are frequently present in patients with autoimmune disease (RA, SS, SLE) and indicate an ongoing active immune process. The presence of secondary vasculitis is much more likely in autoantibody-positive patients than in patients without these antibodies. Of course, the latter findings should be confirmed in a larger cohort of patients.

In RA, SS and SLE antibody response against lamin C was most frequent (52%, 46% and 39% of patients, respectively). Lamin C belongs to the nuclear lamina, which is a meshwork of intermediate filaments associated with the inner nuclear membrane. Besides lamin C three other lamin proteins (A, B1, B2) are known. In RA and SS, our analysis is the first demonstration of lamin C-autoantibodies in a larger collective (33). In addition, anti-lamin autoantibodies were found in other autoimmune diseases (34), especially in SLE (35), autoimmune hepatitis, primary bilary chirrosis and chronic hepatitis delta infection (36). Interestingly, the reactivity of the antibody seems to be restricted to a certain epitope of lamin which is specific for the respective autoimmune disease (37).

The second most frequent antibody response in RA and SS was the response against the nuclear autoantigen NA14. This 14kd protein was initially shown to be located in the nucleus (38). Recent reports demonstrated that NA14 is a centrosomal protein, is also present in other microtubular structures like primary cilia and basal bodies and can mediate functional membrane transport of orphan receptors (39, 40). It is important to note that the coiled-coil domain of NA14 may contribute to its immunogenicity (41), because the domain will favor the formation of self-assembly into fibril-like structures like in other SS-autoantigens pericentrin and NuMA (42, 43). The prevalence of autoantibodies to NA14 in systemic autoimmune disease has so far been studied only in patients from North America and Japan (44). In the latter study, Nozawa *et al*. reported that 14% of patients with primary SS (n=132) are positive for autoantibodies against this protein by ELISA, com-

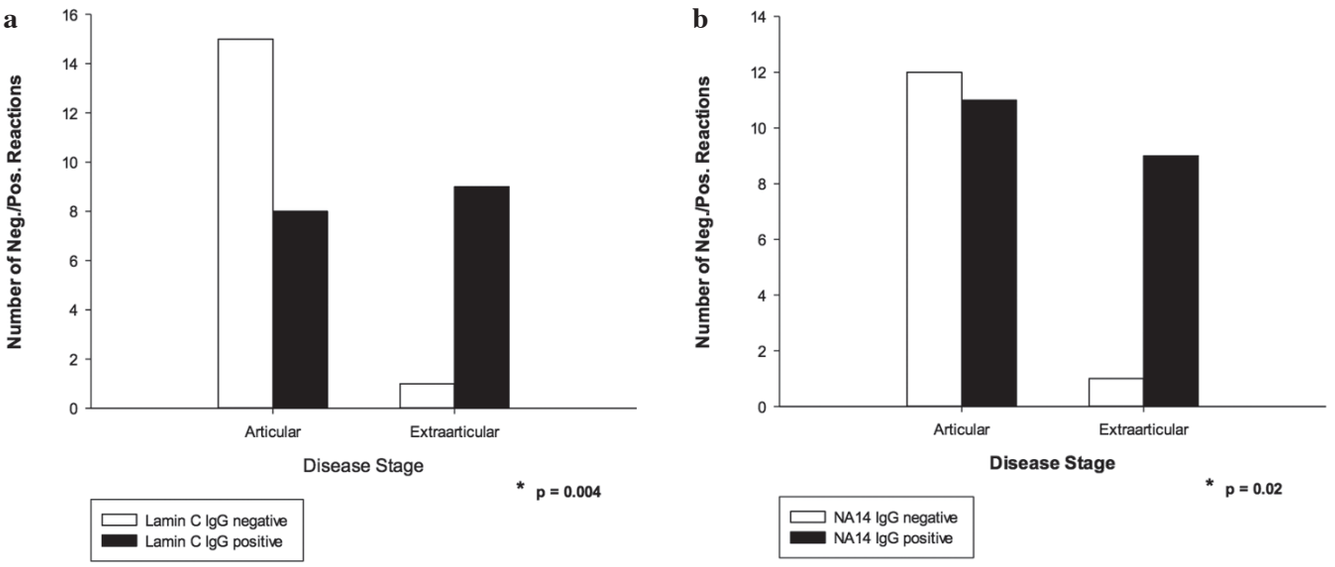

Fig. 7. Autoantibodies against cytoskeletal antigens – Limited *vs*. advanced disease. In patients with RA (n=33) (**a**) lamin C-seropositive and (**b**) NA14-seropositive patients were in a more advanced disease stage in comparison to lamin C-negative and NA14-seronegative patients, because they showed more frequently extraarticular manifestations of the disease (53% and 45%) in comparison with seronegative cases (6% and 8%, p=0,004 and p=0,02, Chi-square test).

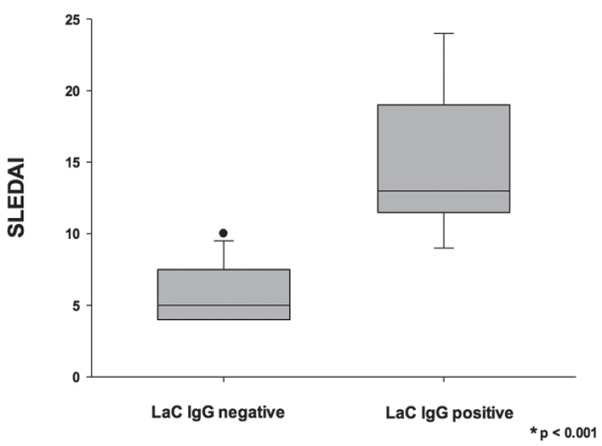

Fig. 8. Autoantibodies against Lamin C and SLEDAI. In SLE (n=23) the occurrence of IgG antibodies against lamin C was associated with the systemic lupus erythematodes activity index (SLEDAI, p<0.001, MWU-test): In lamin C-seronegative (LaC negative) patients a median SLEDAI of 5 was observed, whereas SLEDAI was 13 in lamin C-seropositive patients (LaC positive).

pared to 2% in SLE (n=100) with no antibody response (0%) in RA (n=54). In contrast, we detected in our German patient cohort 82% NA14-antibody-positive patients with SS (n=11) and 61% with RA (n=33). This discrepancy may be caused by different genetic background and/or environmental factors (45-47) as well as by the different techniques used for antibody detection (ELISA, SEREX).

In summary, our study points to a possible association of autoantibodies against laC and CK15 with primary vasculitides, which must be confirmed in a larger cohort of patients. More importantly, it proves that autoantibodies against laC, NA14 and CK15 which are all proteins of the cytoskeleton belong to the characteristic spectrum of autoantibodies in RA, SS and SLE (48). The pathogenic role of these autoantibodies remains speculative (9, 49) and needs further investigations. Because the detection of antibodies against lamin C and NA 14 was associated with advanced disease and secondary vasculitis, their impact on prognosis of the respective disease should be evaluated in prospective studies, ideally using quantitative assays like ELISA

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