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Lupus nephritis and antiphospholipid antibodies

Renal damage in systemic lupus erythematosus (SLE) is mainly due to immune complex--mediated glomerulonephritis. The connection of SLE with antiphospholipid syndrome (APS) leads to significantly worse prognosis of unfavourable progress of the disease (8). APS may be primary (PAPS) or secondary (SAPS), particularly in association with SLE (1, 10). Antiphospholipid antibodies (APA) characteristic features of APS constitute a large and heterogeneous population of autoantibodies directed against different phospholipids or their complexes with plasma proteins (4). The most commonly detected and well known are lupus anticoagulant (LAC), anticardiolipin antibodies (ACL) and anti-ß2 glycoprotein I antibodies (a- ß2 GPI). A large spectrum of renal manifestations has been described in association with APA, such as renal artery stenosis, renal infarction, renal vein thrombosis, acute and chronic thrombotic microangiopathy and more recently the so-called APS nephropathy(APSN) (2, 7). Although there is evidence that APA may expose patients with lupus to an increased risk for vascular thrombosis and pregnancy morbidity, their exact role in pathogenesis of lupus nephritis (LN) is still not clear (7, 8). Lately, cystatin C (Cys C) has been used for detecting patiens with subclinical renal dysfunction (5).

The aim of this study was to determine how frequently ACL and a-ß2 GPI in class IgM and IgG were present in the following groups of patients: 1) with SLE without LN, 2) SLE with LN, 3) SLE with SAPS. We also decided to evaluate the influence of immunosuppressive therapy on the concentration of APA and renal markers in patients with SLE/SAPS and with proteinuria.

MATERIAL AND METHODS

The studied population comprised 41 patients (35 females and six males), mean age 39.2 years ±13.45 (range 19–74) diagnosed according to ACR 1982 (revised 1997) criteria of SLE. Patients were divided into three groups: group 1 – SLE without LN (11 females, mean age 43.5 years ±15.05, age range 22–74,) group 2 – SLE with LN (20 females, mean age 40.1 years ±13.92, age range 19–64), group 3 – SLE and SAPS (four females and six males, mean age 32.9 years ±8.65, age range 24–51). All blood samples from group 2 were taken during an active phase of nephritis (proteinuria with protein greater than 0.5 g/24 and/or hematuria greater than five urinary red blood cells per high-power field and/or impaired renal function-plasma creatinine level greater than 1 mg/dl and/or histologically proven WHO GN (types II–IV). As a control group, ten samples of blood from healthy donors were examined.

In addition, in group of ten patients (six females and four males, mean age 30 years, range 19–46) with SLE and clinical manifestation of APS and with LN in active phase (proteinuria range 300–6596 mg/24 h). Cys C (immunonephelometry method), creatinine (Cr), ACL, a-ß2 GPI in class IgM and IgG were measured. Creatinine clearance (Clcr)-Cockroft-Gault method and activity

index SLEDAI-"renal part" were also assessed before treatment and after two months in these group. These patients were cured with corticosteroids, cyclophosphamide, azathioprine, immunoglobulins, plasmapheresis.

To determine ACL levels we used Cogent standard tests for detection of IgG, IgM, IgA autoantibodies to cardiolipin in human serum. A-ß2 GPI antibodies were detected by ELISA Euroimmun standard test in IgM and IgG class. The data were analyzed by Statistica Visual Basic programme. Most important clinical parameters were calculated by the χ^2 test, U Mann- Whitney where $p<0.05$ was regarded as statistically significant.

RESULTS

In group 1 we have found a significant high level of ACL (>2SD) in six persons (54.5%), median 22.6 u/ml and an insignificant level (<2SD) in three persons (27.2%), median 13,4 u/ml. Two persons had values of ACL below limit for healthy person. The antibodies a-ß2 GPI in class IgM were at a high level in four persons (44.4%), median 61.3 u/ml, a-ß2 GPI in class IgG at a mean level of 7.2 u/ml (all patients had levels of a-ß2 GPI in class IgG below limit for healthy persons). In group 2 we have observed a significant high level of ACL (>2SD) in five persons (25%) median 26.6 u/ml and an insignificant level (< 2SD) in ten persons (50%), median 12.9 u/ml. Five patients (25%) had values of ACL below limit for a healthy person. The antibodies a-ß2 GPI in class IgM were at a high level in seven persons (58.3%), median 40.1 u/ml, a-ß2 GPI in class IgG at mean level 3.1 u/ml (all patients'antibodies were below limit for healthy persons). In group 3 we have found ACL in all patients at a high level, mean 35.1 u/ml. The antibodies a-ß2 GPI in class IgM were at a high level in eight persons (80%), median 70.1 u/ml, a-ß2 GPI in class IgG at a mean level 44.5 u/ml in six patients (60%). Mean levels of ACL, a-ß2 GPI in class IgM and IgG are shown in Figure 1.

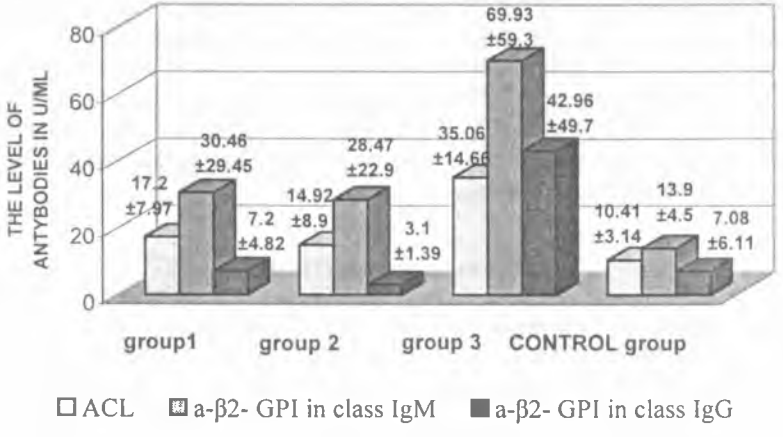

Fig.1. Comparison of ACL, a-ß2 GPI in class IgM and IgG in patients with SLE without LN (group 1), SLE/ LN (group 2), SLE/ SAPS (group 3) and control group

Absolute ACL, a-ß2 GPI in class IgM and IgG levels were compared in the four groups (SLE-SLE/LN-SLE/SAPS-control group). We have found a significantly higher ACL in group 1 and 3 vs control group and a-ß2 GPI in class IgM in group 2 and 3 vs control group and a-ß2 GPI in class IgGin group 3 vs control group. We have noted a higher level of antibodies ACL and a-ß2 GPI in class IgG and IgM in group 3 vs group 1 and 2. We have found a statistically significant positive correlation between ACL and a-ß2 GPI in class IgG in group 3 ($p<0,02$). We have not

noted a statistically significant difference in the prevalence of a- ß2 GPI (class IgM and IgG) and ACL between group 1 and group 2.

Statistics. ACL: group 1 vs group 2 $p>0.05$ and vs group 3 $p<0.007$ and vs control group $p<0.04$; group 2 vs group 3 $p<0.0002$ and vs control group $p>0.05$; group 3 vs control group $p<0.0001$; a- ß2 GPI in class IgM: group 1 vs group 2 $p>0.05$ and vs group 3 $p>0.05$ and vs control group $p>0.05$; group 2 vs group 3 $p<0.05$ and vs control group $p<0.05$; a- ß2 GPI in class IgG: group 3 vs group 1 $p<0.05$ and vs group 2 $p<0.0002$ and vs control group $p< 0.03$. All patients from group 1 and group 2 had levels of antibodies below limit for healthy person.

Levels of antibodies (ACL, a-ß2 GPI in class IgM and IgG), Cr, Clcr, proteinuria and SLEDAI – "renal part" of ten patients with SLE/SAPS and with proteinuria before applied immunosuppressive therapy and after two months of treatment are shown in Table 1.

Table 1. Levels of antiphospholipid antibodies and markers of renal function of ten patients with SLE/SAPS and proteinuria

	Cr mg/dl	Clcr ml/min	CysC mg/l	ACL U/ml	a-ß2 GPI in class IgM U/ml	a-ß2 GPI in class IgM U/ml	SLEDAI renal part	Proteinuria mg
Before treatment	0.94 (±0.27)	97.4 (±34.3)	1.55 (±0.9)	21.4 (±12.5)	53.8 (±43)	20.4 (±31.8)	9 (±8.6)	1730.7 (±2091.8)
Two months later	1.22 (±0.92)	94.35 (±31.9)	1.7 (±1.2)	16.5 (±6.6)	36.4 (±30.2)	16.1 (±20.7)	10 (±8.4)	1198.5 (±1894.4)
Statistical significance	NS	NS	NS	NS	$p<0.02$	NS	NS	NS

We have noted a statistically significant reduction of levels of a-ß2 GPI in class IgM ($p<0.02$) and tendency of reduction in levels of a-ß2 GPI in class IgG and ACL after two months of treatment. We have not found a statistically significant difference between markers of renal function before treatment and two months later, but we have noted a positive correlation between Cys C and SLEDAI- "renal part" ($p<0.05$; $R=0.62$) (Fig. 2).

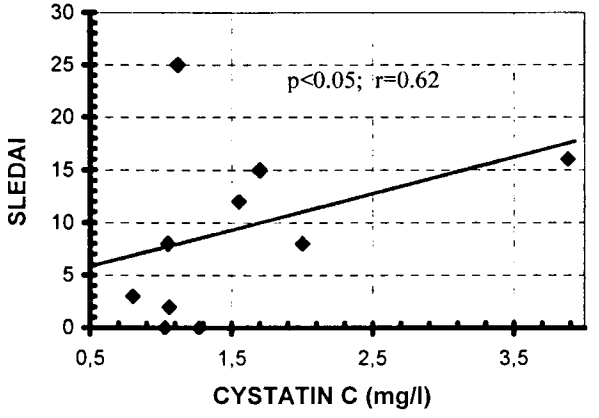

Fig. 2. Correlation between Cys C and SLEDAI-renal part of ten patients with SLE/SAPS and proteinuria

DISCUSSION

In many papers it is emphasized that APA are typical of APS (primary or secondary). In our study we have shown that patients with SLE and SAPS as well as patients with SLE without symptoms of APS have high level of APA serum (the highest ACL and a-ß2 GPI in class IgM). The level of APA was significantly higher in patients with SLE/SAPS. These patients are at a higher risk of thrombosis and fetal loss than patients with SLE without SAPS. It is still unclear what, if any, the impact of APA is on the outcome of LN. Some investigators reported an association between ACL antibody titer and high plasma creatinine level or high proteinuria, but these data were not confirmed by others (3, 6, 7, 9). In our study, there was no difference noted in APA level between patients with SLE and LN and SLE without clinically evident LN.

Two months of intensive immunosuppressive therapy reduce the APA level but this period is too short for improving renal function. Periodical monitoring of APA seems to be a good marker monitoring the efficiency of therapy. Significant dependence between Cys C and SLEDAI makes Cys C a good marker for estimating renal damage in SLE patients.

CONCLUSIONS

1. Patients with SLE/SAPS have the highest level of APA- higher level of APA than patients with SLE without LN and SLE with LN.
2. We have not found any association between APA levels and renal disease in SLE patients.
3. There is a significant dependence between activity of LN estimated by SLEDAI and glomerular filtration measured by Cys C.

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SUMMARY

Lately, in many papers it is emphasised that antiphospholipid antibodies (APA) may expose patients with lupus to an increased risk lupus nephritis (LN). The aim of this study was to determine how frequently anticardiolipin antibodies (ACL) and anti-ß2 glycoprotein I antibodies in class IgM and IgG (a-ß2 GPI in class IgM and IgG) were presented in the following groups of patients: group 1 – with systemic lupus erythematosus (SLE) without LN, group 2 – SLE with LN and group 3 – SLE with secondary antiphospholipid syndrome (SAPS). We also decided to evaluate the influence of intensive immunosuppressive therapy on the concentration of APA and renal markers in patients with SLE/SAPS and with proteinuria. The studied population comprised 41 patients (35 females and six males). All samples from group 2 were taken during an active phase of nephritis. In addition, in ten patients with SLE/SAPS and with LN in active phase (proteinuria range 300–6596 mg/24 h) Cystatin C (Cys C), creatinine (Cr), creatinine clearance (Clcr)-Cockroft -Gault method, ACL, a-ß2 GPI in class IgM and IgG and activity index SLEDAI – "renal part" were measured before treatment and two months later. In group 1 we have found a significant high level of ACL (>2SD) in six persons (54.5%) and an insignificant level (<2SD) in three persons (27.2%). The antibodies a-ß2 GPI in class IgM were at a high level in four persons (44,4%). In group 2 we have found a significant high level of ACL (>2SD) in five persons (25%) and an insignificant level (< 2SD) in ten persons (50%). The antibodies a-ß2 GPI in class IgM were at a high level in seven persons (58.3%). In group 3 we have found ACL in all patients at a high level. The antibodies a-ß2 GPI in class IgM were at a high level in eight persons (80%), a-ß2 GPI in class IgG in six patients (60%). Absolute ACL, a-ß2 GPI in class IgM and IgG levels were compared in all groups, and ACL were found to be significantly higher in group 1 and 3 vs control group, a-ß2 GPI in class IgM in group 2 and 3 vs control group, a-ß2 GPI in class IgG in group 3 vs control group. We have noted a higher level of antibodies ACL and a-ß2 GPI in class IgG and IgM in group 3 vs group 1 and 2. We have found a positive correlation between ACL and a-ß2 GPI in class IgG in group 3 patients ($p<0.02$). We have not noted a statistically significant difference in the prevalence of a- ß2 GPI (class IgM and IgG) and ACL between group 1 and group 2. In ten patients with SLE/SAPS and proteinuria, we have noted a reduction of levels of a-ß2 GPI in class IgM ($p<0,02$) after two months of treatment. We have not found a statistically significant difference between markers of renal function before treatment and two months later, but we have noted positive correlation beetwen Cys C and SLEDAI – "renal part"($p<0.05$; $R=0.62$). We conclude that patients with SLE/SAPS as well as patients with SLE without SAPS have a high level of APA serum.The levels of APA were signifficantly higher in patients with SLE/SAPS. We have not found any association beetwen APA levels and renal disease in SLE patients.

Toczniowe zapalenie nerek a przeciwciała antyfosfolipidowe

Coraz częściej w piśmiennictwie podkreśla się związek między toczniową chorobą nerek (LN) a obecnością przeciwciał antyfosfolipidowych (APA), jednak rola tych przeciwciał nie jest do końca jasna. Celem badania było określenie częstości występowania przeciwciał antykardiolipinowych (ACL) oraz przeciwciał przeciwko ß2 glikoproteinie I w klasie IgM i IgG (a-ß2 GPI w klasie IgM i IgG) w następujących grupach pacjentów: grupa 1 – chorzy z toczniem rumieniowatym układowym (SLE) bez LN, grupa 2 – chorzy z SLE i LN, grupa 3 – chorzy z SLE i wtórnym zespołem antyfosfolipidowym (SAPS). Ponadto ocenialiśmy wpływ terapii immunosupresyjnej na miano APA oraz na markery funkcji nerek u pacjentów z SLE/SAPS i białkomoczem. Badaniami objęto 41 osób (35 kobiet i sześciu mężczyzn). Pacjentów podzielono na trzy grupy: 1) SLE bez LN, 2) SLE z LN, 3) SLE z SAPS. Wszyscy chorzy w grupie 2 mieli aktywną postać LN w czasie badania. Dodatkowo u dziesięciu chorych z SLE/SAPS i białkomoczem (300–6596 mg/dobę) oznaczaliśmy ACL i a-B2 GPI w klasie IgM i IgG, markery funkcji nerek – cystatynę C (Cys C), kreatyninę (Cr), klirens kreatyniny (Clcr), białkomocz dobowy oraz indeks aktywności choroby SLEDAI–część nerkową przed rozpoczęciem leczenia immunosupresyjnego i w dwa

miesiące później. W grupie 1 stwierdziliśmy znacznie podwyższone miano ACL (>2SD) u sześciu osób (54,5%) i nieznacznie podwyższone (<2SD) u trzech osób (27,2%). A-B2 GPI w klasie IgM były podwyższone u czterech chorych (44,4%).W grupie 2 stwierdziliśmy znacznie podwyższone miano ACL (>2SD) u pięciu osób (25 %) i nieznacznie podwyższone (<2SD) u dziesięciu osób (50%). A-B2 GPI w klasie IgM były podwyższone u siedmiu pacjentów (58,3%). W grupie 3 wszyscy pacjenci mieli podwyższone ACL. A-B2 GPI w klasie IgM były podwyższone u ośmiu pacjentów (80%) i a-B2 GPI w klasie IgG u sześciu (60%). Na podstawie analizy statystycznej stwierdziliśmy znacząco wyższe miana ACL w grupie 1 i 3 względem grupy kontrolnej, a-B2 GPI w klasie IgM statystycznie wyższe w grupie 2 i 3 względem grupy kontrolnej, a w klasie IgG wyższe w grupie 3 względem grupy kontrolnej. Stwierdziliśmy również znacząco wyższe miana ACL, a-B2 GPI w klasie IgM i IgG w grupie 3 w stosunku do grupy 1 i 2. W grupie 3 znaleźliśmy dodatnią korelację pomiędzy ACL i a-B2 GPI w klasie IgG ($p<0,02$). Nie było istotnych statystycznie różnic w stężeniach ACL i a-B2 GPI w klasie IgM i IgG pomiędzy grupą 1 i 2. U dziesięciu pacjentów z SLE/SAPS i białkomoczem wykazaliśmy znamienny statystycznie spadek poziomu a-B2 GPI w klasie IgM ($p<0,02$) po dwóch miesiącach leczenia. Nie znaleźliśmy istotnie statystycznych różnic w poziomach markerów funkcji nerek po leczeniu, ale obserwowaliśmy dodatnią korelację pomiędzy Cys C i SLEDAI ($p<0,05$; $R=0,62$). Wykazaliśmy, że pacjenci z SLE/SAPS jak i pacjenci z SLE bez SAPS mają podwyższone miana APA w surowicy. Stężenia APA były znacząco wyższe u pacjentów z SLE/SAPS. Nie wykazaliśmy związku pomiędzy poziomami APA a uszkodzeniem nerek w SLE.