Glycoconjugate Journal (1995) **12**: 241–247

Glycoforms of serum α1-acid glycoprotein as markers of inflammation and cancer

ANDRZEJ MACKIEWICZ* and KRYSTYNA MACKIEWICZ
Department of Cancer Immunology, Chair of Oncology, University School of Medical Sciences at Great Poland Cancer Center, Garbary 15, 61866 Poznań, Poland

Received 7 November 1994

α1-acid glycoprotein (AGP) is a serum acute phase glycoprotein which possesses five N-linked complex type heteroglycan side chains which may be present as bi-, tri- and tetraantennary structures. Depending upon the content of biantennary structure on AGP, up to four glycoforms of AGP are present in serum. These glycoforms can be easily estimated in body fluids by means of crossed affinity-immunoelectrophoresis (CAIE) with the lectin, Concanavalin A (Con A). Con A selectively binds biantennary structures; the more biantennary structures on AGP, the stronger the binding. In acute inflammation, a relative increase of AGP glycoforms with biantennary units is observed - a type I glycosylation change. In some chronic inflammatory states there is an relative decrease of AGP glycoforms with biantennary heteroglycans – a type II glycosylation change. Moreover, in certain other states such as pregnancy, estrogen administration or liver damage, type II glycosylation changes are also seen. A detailed analysis of the clinical applications of the assessment of AGP glycoforms in sera of patients with rheumatic diseases, AIDS and various types of cancers is presented. Accumulated data shows that AGP glycoforms may be very useful in the detection of intercurrent infections in the course of rheumatoid arthritis, systemic lupus erythematosus, or myeloblastic leukaemia, and in the detection of secondary infections in human immunodeficiency virus infected individuals. AGP glycoforms are also very useful in differentiation between various forms of trophoblastic disease and are helpful in monitoring the treatment of these patients. Finally, AGP glycoforms provide valuable information for differentiation between primary and secondary liver cancer.

Keywords: α1-acid glycoprotein, glycoforms, inflammation, cancer, microheterogeneity

Introduction

α1-acid glycoprotein (AGP) is a normal plasma glycoprotein that belongs to a group of proteins referred to as the acute phase proteins (APP) [1]. Forty-three per cent of its molecular mass is composed of carbohydrate moieties. AGP possesses five N-linked complex type heteroglycans which may be present as bi- tri- or tetraantennary structures [2]. AGP concentration may increase by two- to five-fold in the course of a host response (acute phase response) to tissue injury, infection, various inflammatory processes or tumour growth. AGP is a second-phase acute phase protein, since its serum concentration increases about 48 h following tissue injury (compared to a few hours for first-phase acute phase proteins); this change reaches a maximum after 3–4 days and slowly declines during the next 10–14 days [1]. Determination of the levels of a number of acute phase proteins, especially C-reactive protein (CRP), in serum has proven to be very useful in the management of patients with a large variety of pathological conditions. However, there are certain limits to their clinical applications since elevated levels can be seen in both acute and chronic inflammatory states. Examples include, flare-up of some rheumatic diseases, and intercurrent infections in the course of these diseases [3, 4]. Accordingly, such instances create a problem of differential diagnosis which is very often crucial for therapeutic decisions.

Another feature of the acute phase response is the alteration of the N-glycosylation of acute phase proteins and this is most marked in AGP [5–17]. These changes are referred to as microheterogeneity [18]. Two types of microheterogeneity have been distinguished: major microheterogeneity [1, 5] – which reflects changes in the number of branches on N-linked glycans and minor microheterogeneity [19], which occurs through variation in the sialic acid or fucose content. Two different types of change in major microheterogeneity of APP are observed in patient sera. Type I characterized by an increase in the relative amount of biantennary glycans over more branched glycans and this is seen in patients with 'acute' inflammatory processes, and type II characterized by an

*To whom correspondence should be addressed.

increase in the relative amount of tri- and tetraantennary glycans over biantennary glycans; this type has been seen in patients with a number of chronic inflammatory states [1, 12, 15]. However, there are some exceptions to the above classification. In some disorders such as systemic lupus erythematosus (SLE), altered glycosylation of APP is not seen when there is an increase in concentration [3]. Moreover, type II glycosylation changes in AGP are seen in situations not linked to inflammation but rather connected with hyperestrogenism (estrogen administration, pregnancy or liver disorders [7, 13, 21]).

The major microheterogeneity of AGP in biological fluids may be analysed without any purification steps using agarose crossed affinity immunoelectrophoresis (CAIE) with the lectin Concanavalin A (Con A) [22–24]. Con A separates glycoforms of AGP that have various amounts of biantennary glycans [25]. Forms possessing tri- and/or tetraantennary heteroglycans do not react with the lectin in a first dimension gel; forms having one biantennary unit react weakly; forms having two biantennary structures react strongly and so on [2]. Application of specific antibodies against AGP in a second dimension gel allows the detection of these forms in protein mixtures such as serum, ascites or joint fluid. This method gives four glycoforms of AGP: 0, nonreactive with Con A; 1, weakly reactive with Con A; 2, reactive; and 3, strongly reactive with Con A. In order to express the results in a form which can be used to compare different samples the following procedure was introduced into the method. The area under the precipitated peaks corresponding to a particular glycoform was determined by planimetry and the relative amounts of different forms were expressed as percentages of the total. A reactivity coefficient (RC) of Con A-AGP, for each sample was calculated according to the formula: (sum of all lectin reactive variants)/(lectin nonreactive variant). An increase in the RC value corresponds to the type I alteration whereas a decrease corresponds to the type II change.

Originally, Nicollet *et al.* [6] showed increased reactivity of AGP with Con A in sera from four patients with 'acute inflammatory states'. Raynes [7] made a similar observation in sera of patients with sepsis and with acute pancreatitis, and showed that other acute phase proteins such as α1-proteinase inhibitor, α1-antychymotrypsin or ceruloplasmin similarly demonstrated increased Con A reactivity. Subsequently, the same type of glycosylation change was seen in other acute inflammatory processes such as burns [26, 27], infected newborn [28], acute mediastinitis [29], AIDS [30], intercurrent infection in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [3, 4]. In contrast, in patients with active RA, ankylosing spondylitis (AS) or polymyalgia rheumatica, decreased AGP-Con A reactivity was found [4, 10, 11, 17]. Similarly, others [8, 9] observed a decrease in AGP-Con A reactivity in sera of patients with chronic inflammatory processes, including ulcerative colitis and Crohn's disease [8] or chronic bacterial infections [16].

Based on the data obtained in my laboratory and many years of experience investigating this topic we will present in this review some of the potential clinical applications of the determination of major microheterogeneity (glycoforms) of AGP in disease. For comparison, results will be shown for the determination of CRP in the same patient sera [31].

The RC and CRP values were compared by the Mann-Whitney test. Sensitivity and specificity of AGP microheterogeneity and CRP determinations for clinical diagnosis were calculated according to Sox and Liang [32]. Correlation between RC and disease activity grade were determined using Spearman's rank correlation coefficient.

AGP glycoforms in sera of healthy individuals

Sera obtained from 102 healthy individuals were used as reference population. CAIE in all these sera showed three glycoforms of AGP, 0, 1 and 2. The RC value was 1.34 ± 0.02.

Rheumatic diseases

Proper evaluation of a patient with RA, SLE or AS depends upon having objective and reproducible indicators of the disease activity, and the effectiveness of the therapy should be judged by its ability to control the activity. An additional clinical problem which appears during the normal course of rheumatic diseases, is intercurrent infection. It is firmly established that there is a higher rate of mortality among patients with RA than seen in the general population because of accompanying infections [33]. This increased mortality rate is not the result of a higher frequency of infections, but is rather, the result of a more severe course for the infection that normally occur [34]. Thus, the ability to differentiate an intercurrent infection from RA in these patients is of great clinical value in the management of the disease. Similarly, in SLE, intercurrent infection with or without fever presents a diagnostic problem. Since fever may be a symptom of active SLE, differentiation of an infection from an exacerbation of disease is often difficult [35]. Intercurrent infection detected in RA or SLE patients leads to the application of the proper therapy, which in most cases is antibiotic treatment. On the other hand, flare-up of the disease requires, in most cases, application of steroids. Since both treatments, if administered in the wrong situatian may have serious consequences for the patient; proper diagnosis of intercurrent infection is crucial.

Clinical and laboratory findings for polymyositis-dermatomyositis in the early course of disease, may overlap with those of polymyalgia rheumatica, the ability to differentiate noncharacteristic clinical features of polymyositis-dermatomyositis from polymyalgia rheumatica is of diagnostic importance.

Sera from 280 rheumatic patients were investigated (75 patients with SLE, 120 patients with RA, 48 patients with AS, 15 patients with polymyalgia rheumatica, five patients with polymyalgia with histological proof of giant cell arteritis, 17 patients with polymyositis-dermatomyositis). At the time of

Table 1. α1-acid glycoprotein-Con A reactivity in sera of patients with rheumatic diseases.

Clinical diagnosis	n	*AGP-RC*[d]	*CRP (median)*[e]
Healthy individuals	102	1.34 ± 0.02	0
Rheumatoid arthritis I	10	1.20 ± 0.20[a]	4
Rheumatoid arthritis II	25	1.09 ± 0.11[a]	20
Rheumatoid arthritis III	34	0.90 ± 0.17[a]	47
Rheumatoid arthritis IV	38	0.71 ± 0.14[a]	63
Rheumatoid + infection	15	1.87 ± 0.23[ab]	60
Ankylosing spondylitis – inactive	14	1.08 ± 0.25[a]	4
Ankylosing spondylitis – active	30	1.01 ± 0.19[a]	14
Ankylosing + peripheral joints	4	0.92 ± 0.20[a]	40
SLE I	20	1.41 ± 0.48	0
SLE II	18	1.40 ± 0.38	8
SLE III	22	1.36 ± 0.40	36
SLE + infection	20	2.81 ± 0.76[ac]	40
Polymyalgia rheumatica	15	0.92 ± 0.17[a]	76
Polymyalgia + arteritis	5	0.91 ± 0.12[a]	68
Polymyositis-dermatomyositis	17	1.38 ± 0.52	6

n, number of sera studied;
[a]Significant difference when compared with healthy individuals.
[b]Significant difference when compared with noninfected RA.
[c]Significant difference when compared with noninfected SLE.
[d]Mean ± SD
[e]mg l^{-1}
Adopted from reference 1 with permission.

blood sampling, the patient's disease activity was assessed according to clinical criteria. Patients with SLE were divided into three subgroups of different disease activity [36]. Subgroup I, involvement of one system and the absence of fever – 20 patients; subgroup II, involvement of one system and the presence of fever, or involvement of more than one system without fever – 18 patients; subgroup III, fever and the involvement of at least two systems – 22 patients. Patients with RA were assigned to one of four subgroups based on a multivariate analysis of disease activity [37]. This comprised morning stiffness, a pain scale, grip strength, articular index, haemoglobin concentration and erythrocyte sedimentation rate (I, inactive disease – 10 patients; II, mildly active disease – 25 patients; III, moderately active disease – 36 patients; IV, severe disease – 38 patients). AS patients were divided into two subgroups: 14 patients with inactive disease and 34 with active disease [38]. From the subgroup of patients with active disease, four patients who had peripheral joints affected (presence of joint fluid) were distinguished, and regarded as a separate subgroup. Polymyositis-dermatomyositis patients were classified into one of four diagnostic categories [39]: I, primary idiopathic polymyositis – two patients; II, primary idiopathic dermatomyositis –eight patients; III, polymyositis with malignancy – two patients; IV, overlap syndromes – five patients. In addition, 15 of the 75 SLE patients and 11 of the 120 RA patients experienced 20 and 15 episodes of intercurrent infection respectively. Infection was diagnosed by a positive bacterial culture in most cases, and by a clinical picture and therapeutic response that was strongly suggestive of infection in few cases.

CAIE revealed from two to four glycoforms of AGP in sera of the patients studied. Two forms were found in a number of patients with severely active RA. Four glycoforms were seen in most of the sera, from infected RA and SLE patients. In the remaining sera, three forms were found.

Mean values of AGP-RC in sera from healthy individuals and patients are shown in Table 1. Comparison of the RC values with activity grades of RA, AS and SLE showed a significant correlation only for RA ($r = -0.75$; $p < 0.001$). There was also a correlation of serum CRP values with grades of RA activity and this gave a correlation coefficient of 0.69. The results show that AGP glycoforms appear to be valuable biochemical indicator of RA activity, however, similar information may be gained from CRP measurement. Moreover, as with CRP, it does not seem likely that the determination of AGP-RC can be used as independent indicator. In AS patients, RC decreased in a similar manner to RA although the change was less. However, no differences between clinically inactive and active patients were seen. Only in patients who had peripheral joints affected was a decrease in RC observed compared to the other AS groups. The results indicate that determination of AGP microheterogeneity may be a useful discriminator of AS but has no value as an indicator of disease activity. In sera of SLE patients, in contrast to RA and AS,

there were no changes in the proportions of AGP glycoform. The RC values were similar to those seen in healthy donors. Similary, in patients with polymyositis- dermatomyositis the RC values were the same as those observed in sera of healthy individuals.

In infected patients with RA and SLE, a significant increase in RC was found. Intercurrent infection in SLE could be diagnosed with sensitivity of 90% and a specificity of 92% when the cutoff RC value was taken as 2.0. In the same patients, high CRP levels (> 60 mg l^{-1}) had poor sensitivity (39%) for predicting infection. Differentiation of RA patients with infection from patients with active RA (grades III and IV) based on RC gave a sensitivity of 82% and a specificity of 91%. CRP levels in these two groups of patients were not significantly different. Although measurement of CRP levels is useful in the assessment of RA activity, it is of limited value in the diagnosis of intercurrent infection in these patients. The determination of CRP might be helpful in differentiating healthy people from RA patients experiencing an infection. When the presence of the fourth AGP variant was considered in the analysis, accompanying infections could be confirmed in all patients with RA and SLE.

An RC above 1.25 was not found in the polymyositis rheumatica patients, but it was seen in sera from 13 of the 17 patients with polymyositis-dermatomyositis (sensitivity 76%; specificity 100%). In 15 of 17 patients with polymyositis-dermatomyositis AGP glycoform three was found (sensitivity 88%; specificity 100%). Accordingly, these differences made it possible to differentiate these diseases. On the other hand, using CRP determination gave a sensitivity of 60% and a specificity of 68%.

Cancer

Several attempts have been made to use AGP glycoform determination for the diagnosis and management of cancer. In an early report Hansen *et al.* [40] claimed they were able to differentiate lung cancer from inflammatory lung disease using AGP glycoforms but this was not confirmed by others [41]. However, other studies of Hansen *et al.* [8, 9] have demonstrated that CAIE of AGP can differentiate between chronic colorectal inflammation (ulcerative colitis, Crohn's disease) and colorectal cancer. Dobryszycka and Katnik [42] have shown that the AGP glycoforms may be helpful in the assessment of the stage of ovarian carcinoma, and in the evaluation of the effectiveness of therapy in stage IV ovarian cancer.

Patients

Serum samples from 364 patients were studied. A wide variability in the distribution of AGP glycoforms was observed, with no cancer specific pattern. Two (0 and 1) to four (0, 1, 2 and 3) glycoforms were seen. In nine patients with cancer (seven of 29 yolk sac tumour, one of 16 hepatoma and one of 46 bile duct cancer), an 'atypical' AGP of low mobility was seen, both in Con A and control experiments (without a lectin). However, in defined groups of patients, there were significant changes toward glycoforms being more or less reactive with Con A. Mean values for AGP-RC in sera from healthy individuals and cancer patients are shown in Table 2.

Table 2. α1-acid glycoprotein glycoforms in sera of cancer patients.

Clinical diagnosis	n	*AGP-RC*[a]
Healthy adults	102	1.34 ± 0.02
Yolk sac tumour	22	1.75 ± 0.67[bd]
Hepatoma	16	0.71 ± 0.46[bc]
Pancreas + bile duct cancers	56	0.91 ± 0.53[bc]
Metastatic liver tumours	20	1.12 ± 0.44[cd]
Benign liver disorders	41	0.84 ± 0.28[b,c]
Cholelithiasis	20	1.21 ± 0.70[cd]
Hydatidiform mole	16	0.51 ± 0.18[b]
Invasive mole	25	0.92 ± 0.20[b,e]
Choriocarcinoma	16	1.23 ± 0.36[ef]
Myeloblastic leukaemia		
Remission	26	1.43 ± 0.45
Relapse	27	1.61 ± 0.54
Remission + infection	7	2.22 ± 0.29[bgh]
Relapse + infection	32	1.98 ± 0.58[bgh]

n, number of patients studied.
[a]Mean ± SD.
[b]Significant difference when compared with healthy adults.
[c]Significant difference when compared with yolk sac tumour.
[d]Significant difference when compared with hepatoma.
[e]Significant difference when compared with hydatidiform mole.
[f]Significant difference when compared with invasive mole.
[g]Significant difference when compared with remission of leukaemia.
[h]Significant difference when compared with relapse of leukaemia.
Adopted from reference 1 with permission.

Tumours of the digestive system

Sera from 94 patients with tumours of the digestive system (46 patients with cancers of bile duct, 11 with pancreatic cancers, 17 with hepatoma, and 20 with metastatic liver tumours) and from 41 patients with inflammatory disorders of the liver and bile ducts were studied. Metastatic tumours originated in the lungs in five patients, in the stomach in four patients, in the large bowel in five patients, in the breast in two patients and in the ovaries in four patients. Benign liver disorder included liver cirrhosis – 16 patients, viral hepatitis – 25 patients, and bile duct obstruction (cholelithiasis) resulting in periportal fibrosis – 20 patients.

In cancers of the digestive system – originating in the liver, pancreas or bile duct there is a decrease in serum AGP-Con A reactivity, whilst in metastatic liver tumours such changes are not seen. This diversity could be used to differentiate between primary and secondary liver cancer (sensitivity 80%, specificity 62%). In patients with liver cirrhosis and patients with viral hepatitis similar changes were observed as in hepatoma patients. However, in patients with bile duct

obstruction, AGP glycoform distribution was similar to that seen in healthy individuals. This indicates that differentiation between cancer and liver cirrhosis is impossible, but suggests that this test might be helpful in differentiation between cholelithiasis, hepatoma and liver cirrhosis.

Yolk sac tumour

Sera from 29 patients with yolk sac tumour were studied. Cancer originated in 23 cases in the testes, in three cases in the ovaries and in three other cases in retroperitoneal tissue. Sera were collected at the time of the first contact with the physician and were alpha-fetoprotein positive.

A shift of serum AGP glycoforms toward forms more reactive with Con A was found. RC in sera of these patients was significantly higher than in patients with cancer of the liver (both primary and secondary), as well as in patients with benign liver disorders. Accordingly, determination of AGP-RC might be helpful in the differentiation of these types of cancers, especially when the AFP serum levels are high.

Trophoblastic diseases

Persistent trophoblastic disease is present in a number of clinical forms. However, from a practical point of view, three major pathological forms were distinguished: hydatidiform mole, invasive mole and choriocarcinoma. The major clinical problems of this disease are the early diagnosis of progression from hydatidiform mole through invasive mole to choriocarcinoma; selection of patients which require chemotherapy; and evaluation of responsiveness to chemotherapy. Measurement of β-human chorionic gonadotropin (β-HCG) is very useful in the management of this disease, however, early differentiation between the three forms of trophoblastic disease based on β-HCG is impossible.

Sera from 57 women with trophoblastic disease were studied. Sixteen patients had histologically confirmed hydatidiform mole, 25 patients had a clinical form of invasive mole but only half of these patients had histological examination which demonstrated proliferative mole. In the other half of the patients the presence of choriocarcinoma could not be ruled out. In 16 patients, choriocarcinoma was diagnosed. Hydatidiform mole was treated by surgery or methotrexate (MTX) chemotherapy as required. Invasive mole and choriocarcinoma were treated by either a single cytotoxic agent or with multiple agents.

In hydatidiform mole there was a dramatic decrease in serum AGP-Con A reactivity. Similar changes, but to a lesser extent, were also seen in patients with invasive mole, while in patients with choriocarcinoma the mean RC values were no different from those seen in healthy individuals (Table 2). Parallel studies of β-HCG in patient sera demonstrated significant overlap in the values between the groups. In patients with hydatidiform mole, successful surgical removal of the mole resulted in the return to normal RC values. In patients with an invasive mole where chemotherapy was successful, the RC returned to normal values. When the invasive mole was resistant to chemotherapy, RC did not change. Correlation between β-HCG and RC was not observed. The conclusions are, that determination of AGP glycoforms might serve as a valuable test for the differentiation between three forms of trophoblastic disease. Moreover, it may provide additional information during the treatment of the disease.

Acute myeloblastic leukaemia

Sera from 92 patients with acute myeloblastic leukaemia (33 patients during remission, 59 patients during relapse) were studied. In seven of the patients with the remission and in 32 of the relapse patients, intercurrent infections were observed.

In patients with acute myeloblastic leukaemia, distribution of AGP glycoforms (0, 1, 2) was similar to that observed for healthy individuals. However, intercurrent infections caused a significant shift towards more AGP-Con A reactive glycoforms. In the majority of these patients a fourth glycoform (peak 3) was seen. Determination of RC could therefore be useful for the detection of intercurrent infections in leukaemia (sensitivity 69%, specificity 78%) and inclusion of glycoform 3 in the analysis significantly increased sensitivity (92%) and specificity (100%). However, serum CRP measurement provided good discrimination between these two groups of patients (sensitivity 100%, specificity 89%).

Aids

Sera from 109 HIV infected patients were analysed. When the blood samples were taken, patients were assigned into one of four groups according to the Centers for Disease Control, US Department of Health and Human services classification

Table 3. α1-acid glycoprotein glycoforms in sera of HIV infected patients.

Group[a]	n	*AGP-RC*[bd]	*CRP*[c]	
			mean ± SD	*median*
I	1	1.24	18	18
II	22	1.53 ± 0.68	1.4 ± 3.5	0
III	23	1.4 ± 0.36	4.2 ± 6.9	0
IV a–no joints	4	1.45 ± 0.48	0	0
IV a–joints	3	0.76 ± 0.23	1.5 ± 1.5	0
IV c_1–no joints	20	2.21 ± 0.87	9.7 ± 16	4
IV c_1 + joints	9	1.19 ± 0.21	3.9 ± 6.2	0
IV c_2–no joints	8	1.68 ± 0.28	4.9 ± 4.6	0
IV c_2 + joints	6	1.27 ± 0.2	23.8 ± 46	5
IV d–no joints	9	1.49 ± 0.31	35 ± 48	12
IV d + joints	4	0.96 ± 0.21	21.4 ± 32	6

n, number of patients studied.
[a]CDC stage (ref. 43)
[b]Mean ± SD.
[c]mg l^{-1}.
[d]See text for relevant statistical comparisons.

(Atlanta, Georgia) [43]: *group I* (acute infection) – 1 patient, *group II* (asymptomatic infection) – 22 patients; *group III* (persistent generalized lymphadenopathy) – 23 patients; *group IV* (subgroups a, c_1, c_2, d) (other diseases) – 63 patients. In 22 patients of group IV, joints were affected (14 patients had Reiter's syndrome, six psoriatic arthritis and two chronic idiopathic arthritis. Five of 20 patients of group IV c_1 with no arthritis had pneumocystitis carinii pneumonia (PCP), one toxoplasmosis, eight systemic fungal infection, and six systemic mycobacterial infection. Six of group IV c_1 with arthritis had PCP and three had cytomegalovirus infection. In group IV c_2 with no arthritis, five had oral candidiasis and three had multidermal herpes zoster. In group IV c_2 patients with arthritis, four patients had oral candidiasis, one oral candidiasis and tuberculosis and one oral hairy leukoplakia.

The RC values of patients assigned to groups I, II, III were not significantly different from normal. However, in group IV, RC values (depending on the presence or absence of arthritis) differed significantly from those observed in healthy subject, and groups I–III. In group IV without arthritis, a significant increase in RC ($p < 0.016$ compared with healthy individuals) was observed (Table 3). In contrast, in group IV patients with arthritis, a significant decrease of RC was seen ($p < 0{,}001$ compared with healthy individuals and $p < 0.021$ compared with groups I–III). Within group IV, there was a significant difference in RC values between patients with and without arthritis ($p < 0.001$). In group IV c_1 patients without arthritis, especially in those who had *PC*P infection, a dramatic increase of RC was found (RC = 2.72 ± 0.43). When the RC cutoff value of 1.9 was selected, secondary infection could be detected with a specificity of 62% and a sensitivity of 96%, and PCP with a specificity of 100% and a sensitivity of 96%. Similar evaluation of CRP measurements (cutoff value, 10 mg l^{-1}) gave a sensitivity of 32% and a specificity of 85% (for PCP 20% and 85%, respectively). In addition, in patients who developed chronic liver diseases such as liver cirrhosis or chronic hepatitis, a decrease of RC was also found. According to the previously described classification of glycosylation changes, the alterations in AGP glycoforms of HIV infected patients were type I changes in patients with secondary infectious diseases and type II changes in patients who had affected joints.

The high specificity and sensitivity of RC determination for the detection of secondary infection in AIDS implies that it is useful clinically. However, when evaluating the results one has to consider other factors, such as joint involvement. Sera of patients analysed in these studies were obtained mainly from two centres in the USA [30]. Cleveland Clinic Foundation, Cleveland, Ohio and Louisiana State University Medical Center, New Orleans, Louisiana. Interestingly, in a large number of HIV-infected individuals in Poland, liver damage caused by hepatitis B and/or C virus infection and liver cirrhosis was found (data not published). As demonstrated by us (Table 2) and by others [7, 13, 20], in liver disease AGP-RC values are significantly lower than normal. When evaluating results, therefore, liver damage as well as joint involvement must be taken into consideration.

Acknowledgements

Supported by KBN Grants 41121 and 41076.

References

1. Kushner I, Mackiewicz A (1993) *Acute Phase Proteins: Molecular Biology, Biochemistry and Clinical Applications* (Mackiewicz A, Kushner I, Baumann H, eds) pp. 4–19. Boca Raton: CRC Press.
2. Bierhuizen M, De Wit M, Govers C, Ferwerda W, Koeleman C, Pos O, Van Dijk W (1988) *Eur J Biochem* **175**:387–94.
3. Mackiewicz A, Marcinkowska-Pieta R, Ballou S, Mackiewicz S, Kushner I (1987) *Arthritis Rheum* **30**:513–18.
4. Pawlowski T, Mackiewicz S, Mackiewicz A (1989) *Arthritis Rheum* **32**:347–51.
5. Van Dijk W, Turner GA, Mackiewicz A (1994) *Glycosyl Dis* **1**, 5–14.
6. Nicollet I, Lebreton JP, Fontaine M, Hiron M (1981) *Biochim Biophys Acta* **668**:235–45.
7. Raynes J (1982) *Biomedicine* **36**:77–86.
8. Hansen JE, Jensen SP, Nørgaard-Pedersen B, Bøg-Hansen TC (1986) *Electrophoresis* **7**:180–86.
9. Hansen JE, Iversen J, Lihme A, Bøg-Hansen TC (1987) *Cancer* **60**:1630–35.
10. Mackiewicz A, Pawlowski T, Mackiewicz-Pawlowska A, Wiktorowicz K, Mackiewicz S (1987) *Clin Chim Acta* **163**:185–90.
11. Mackiewicz A, Khan MA, Reynolds TL, Van der Linden S, Kushner I (1989) *Ann Rheum Dis* **48**:99–103.
12. Breborowicz J, Mackiewicz A (1989) *Electrophoresis* **10**:568–73.
13. Jezequel M, Seta NS, Corbic MM, Feger JM, Durand GM (1988) *Clin Chim Acta* **176**:49–57.
14. Hachulla E, Laine A, Hayem A (1988) *Clin Chem* **34**:911–13.
15. Mackiewicz A, Pawlowski T, Wiktorowicz K, Mackiewicz S (1986) In *Lectins: Biology, Biochemistry, Clinical Chemistry*, Vol. V (Bøg-Hansen TC, van Driessche E, eds) pp. 623–630. Berlin, New York: de Gruyter & Co.
16. Fassbender K, Zimmerli W, Kissling R, Sobieska M, Aeschlimann A, Kellner M, Muller W (1991) *Clin Chim Acta* **203**:315–21.
17. Pawlowski T, Aeschlimann A, Kahn MF, Vaith P, Mackiewicz SH, Mueller W (1990) *J Rheumatol* **17**:1187–92.
18. Hatton MWC, Marz L, Regoeczi E (1983) *Trends Biochem Sci* **92**: 287–91.
19. De Graaf TW, Van der Stelt ME, Anbergen WG, Van Dijk W (1993) *J Exp Med* **177**:657–66.
20. Serbource-Goguel Seta N, Durand G, Corbic M, Agneray J, Feger J (1986) *J. Hepatol* **2**:245–52.
21. Wells C, Bøg-Hansen TC, Cooper EH, Glass MR (1981) *Clin Chim Acta* **109**:59–67.
22. Bøg-Hansen TC (1973) *Analyt Biochem* **56**:480–88.
23. Mackiewicz A, Mackiewicz S (1986) *Analyt Biochem* **156**:481–88.

24. Mackiewicz A, Breborowicz J (1992) In *Affinity Electrophoresis: Principles and Application* (Breborowicz J, Mackiewicz A, eds) pp. 119–33. Boca Raton: CRC Press.
25. Narasimhan S, Freed JC, Schachter H (1986) *Carbohydr Res* **149**:65–83.
26. Pos O, van der Stelt ME, Wolbink G-J, Nijsten MW, van der Tempel DL, van Dijk W (1990) *Clin Exp Immunol* **82**:579–82.
27. Pawtowski T, Biczysko M, Solarewicz M, Mackiewicz S (1988) In *Lectins: Biology, Biochemistry, Clinical Chemistry*, Vol 6 (Bog-Hansen TC, Fried J, eds) pp. 491–96. St Louis: Sigma Chemical.
28. Seta N, Lebrun D, de Crepy A, Feger J, Durand G (1992) In *Affinity Electrophoresis: Principles and Application* (Breborowicz J, Mackiewicz A, eds) pp. 183–87. Boca Raton: CRC Press.
29. Feger J, Seta N, Giai-Brueri M, Durand G (1992). In *Affinity Electrophoresis: Principles and Application* (Breborowicz J, Mackiewicz A, eds) pp. 257–62. Boca Raton: CRC Press.
30. Mackiewicz A, Khan MA, Górny A, Kapcińska M, Juszczyk J, Calabrese LH, Espinosa LR (1994) *J Inf Dis* **169**:1360–63.
31. Mackiewicz A, Wiktorowicz K, Mackiewicz S (1985) *Arch Immunol Ther Exp* **33**:693–710.
32. Sox Jr HC, Liang MH (1986) *Ann Intern Med* **104**:515–23.
33. Baum J (1971) *Arthr Rheum* **14**:135–37.
34. Vanderbroucke JP, Kaaks R, Valkenburg HA, Boersma JW, Cats A, Festen JJM, Hartman AP, Huber-Bruning O, Rasker JJ, Weber J (1987) *Arthritis Rheum* **30**:810–13.
35. Rothfield N (1985) In *Textbook of Rheumatology*, 2nd edn (Kelley WN, Harris ED, Ruddy S, Sledge CB, eds) pp. 1070–132. Philadelphia: WB Saunders.
36. Rothfield NF, Pace N (1962) *N Engl J Med* **266**:535–38.
37. Mallya RK, Mace BEW (1981) *Rheumatol Rehab* **20**:14–17.
38. Van der Linden S, Ferraz MB, Tugwell P (1990) In *Ankylosing Spondylitis and Related Spondyloarthropaties*, in *Spine*, Vol 4, (Khan MA, ed.) pp. 583–611. Philadelphia: Hanley & Belfus, Inc.
39. Hunder GG, Hazleman BL. Giant cell artheritis and polymyalgia rheumatica. In *Textbook of Rheumatology*, 2nd edn (Kelley WN, Harris ED, Ruddy S, Sledge CB, eds) pp. 1166–93. Philadelphia: WB Saunders.
40. Hansen JES, Larsen VA, Bøg-Hansen TC (1984) *Clin Chim Acta* **138**:41–47.
41. Bleasby AJ, Knowles JC, Cooke NJ (1985) *Clin Chim Acta* **150**:231–35.
42. Dobryszycka W, Katnik I (1992) In *Affinity Electrophoresis: Principles and Application* (Breborowicz J, Mackiewicz A, eds) pp. 211–25. Boca Raton: CRC Press.
43. Centers for Disease Control. US Department of Health and Human Services (1986) *Ann Inter Med* **105**:234–37.