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December 12/03:

Autoantibodies prior to Clinical Onset of SLE

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that, apart from its clinical manifestations, goes along with the production of several characteristic autoantibodies. Anti-dsDNA and anti-Sm antibodies are highly disease-specific, whereas anti-SSA(Ro) and anti-SSB(La) antibodies may also occur in Sjogren's syndrome. Anti-nuclear ribonucleoprotein antibodies are found in SLE and are characteristic for Mixed Connective Tissue Disease (MCTD) but they may also occur in other diseases, such as Sjogren's syndrome, scleroderma, rheumatoid arthritis etc. Phospholipid antibodies are the hallmark of the antiphospholipid syndrome (APS) that may present as secondary form together with SLE. These facts are well known and established.

The temporal relationship between the development of these antibodies and onset of clinical disease, however, is largely unknown as patients' serum samples are in general not available prior to disease onset. There is only one study concerning dsDNA antibodies that deals with their production years before onset of SLE (see Publication of the Month 12/01).

In the following recent publication, the development of all relevant autoantibodies was studied prior to the onset of clinical symptoms of SLE in 130 selected patients:

Arbuckle MR, McClain MT, Rubertone MV et al. (2003)
Development of Autoantibodies before the Clinical Onset of Systemic Lupus Erythematosus
N Engl J Med 349, 1526 - 1533

Out of 30 million serum specimen collected from 5 million military personnel, 130 patients with SLE were selected. For these patients, serum samples prior to disease onset were available, at a mean of 4.4 +/- 2.5 years before onset (with a maximal interval of 9.4 years). In 115 of the 130 SLE patients (88 %) at least one autoantibody was present before the diagnosis. Antinuclear antibodies (ANA) were present in 78 % (at a dilution of at least 1:120 on HEp-2000 cells), anti-dsDNA in 55%, anti-Ro in 47 %, anti-La in 34 %, anti-Sm in 32 %, anti-nuclear ribonucleoprotein in 26 % and anti-phospholipid antibodies in 18% of all patients (see Table 1, p. 1528). ANA, anti-phospholipid, anti-Ro and anti-La antibodies preceded anti-Sm and anti-nuclear ribonucleoprotein antibodies (a mean of 3.4 years vs. 1.2 years before diagnosis)(see Table 1, p. 1528). dsDNA Antibodies occurred in the intermediate term (2.2 years before diagnosis). As in many cases (90 of 130 patients, or 69 %) the earliest available serum sample was already positive, these measurements are underestimates of the time from the development of antibodies to the diagnosis. Of the 130 initially matched controls, only 3.8 % were positive for at least one autoantibody.

Six years before the diagnosis, patients had, on average, 1.47 different autoantibodies out of the 7 analysed. This number increased to 2.58 with the appearance of the first clinical criterion and then to 3.01 at diagnosis. Five years after diagnosis, patients presented with, on average, 3.07 different specificities.

These results are unique and very interesting. They reveal that the development of autoantibodies is associated with the disease processes leading to SLE. Nevertheless, it is an open issue if autoantibodies in SLE are really pathogenic or if they represent epiphenomena of the disease process.

The authors estimate that the presence of ANA, anti-Ro, anti-La, or antiphospholipid antibodies increases the risk for SLE by a factor of at least 40. On the other hand, the onset of clinical illness is not immanent and the publication does not address the question if autoantibody positive asymptomatic persons should be monitored or treated.

November 11/03:

Antiphospholipid Syndrome

Antiphospholipid syndrome (APS) is in most cases diagnosed according to the International Consensus Statement from 1999. Accordingly, diagnosis is based on clinical features and on laboratory criteria: the patient must fulfill at least one of the clinical criteria (vascular thrombosis or pregnancy morbidity) and at least one of the laboratory criteria (anticardiolipin antibodies of immunoglobulin IgG and/or immunoglobulin M isotype in blood, present in medium or high titer on two or more occasions, at least 6 weeks apart, measured by a standardized ELISA for beta2-glycoproprotein I dependent cardiolipin antibodies or lupus anticoagulant antibodies).

Due to the fact that APS has evolved to be a highly systemic disease, Y. Shoenfeld has proposed to change its name to "systemic (Hughes') antiphospholipid syndrome":

Shoenfeld Y. (2003)
Systemic antiphospholipid syndrome
Lupus 12, 497 - 498

Although the term "antiphospholipid syndrome" was introduced already in 1986, several aspects of APS (including clinical features and diagnosis, therapeutic approaches and pathogenesis) still remain unresolved. The following recent review highlights the most important controversies related to obstetric complications of the APS:

*Branch DW, Khamashta MA (2003)****Antiphospholipid Syndrome: Obstetric Diagnosis, Management and Controversies***
Obstet Gynecol 101, 1333 - 1344

Open items of APS as discussed in this review are the following: 1. Detectable antiphospholipid antibodies are found in up to 5 % of apparently healthy controls and up to 35 % of SLE patients. The prospective risks of a positive test for antiphospholipid antibodies in otherwise healthy subjects are unknown. 2. Although administration of human antiphospholipid antibodies results in clinical manifestations in several animal models, the underlying mechanism remains unsolved. Probable is the interaction of circulating antiphospholipid antibodies with phospholipids expressed by disturbed cells, such as activated platelets. 3. The substantial interlaboratory variability for anticardiolipin antibodies remains a serious problem. 4. Studies regarding therapy often lead to conflicting results, mainly due to differences in patient selection (both clinical and laboratory) 5. The best therapy of a subsequent pregnancy after recurrent pregnancy losses despite treatment with heparin is at present not known.

October 10/03:

Glomerular Immune Deposits in Patients with Systemic Lupus Erythematosus

About 50 % of patients with systemic lupus erythematosus (SLE) have clinical evidence of kidney involvement, manifested by proteinuria and abnormalities of urinary sediment. One of the major events leading to kidney involvement is the formation of glomerular immune deposits that initiates glomerular injury and the subsequent loss of renal function. To understand which are the immune processes participating in the formation of glomerular immune complexes, it is important to find out their composition. Several attempts have already been undertaken to recover and analyze antibodies from glomerular immune deposits, with varying success.

In the following current publication, extracts from glomerular basement membrane fragments were tested for IgG antibodies to 14 different antigens:

Mannik M, Merrill CE, Stamps LD, Wener MH (2003)
Multiple Autoantibodies Form the Glomerular Immune Deposits in Patients with Systemic Lupus Erythematosus
J Rheumatol 30, 1495 - 1504

Kidney tissue from 23 SLE patients was analysed after autopsy. Glomeruli were isolated and the glomerular basement membrane fragments were extracted either with a pH 2.5 buffer or with 6 M guanidinium hydrochloride. Extracted IgG and IgG antibodies to 14 different antigens were quantified by capture assays.

Antibodies to dsDNA, the collagen-like region of C1q, Sm, SSA(Ro), SSB (La) and chromatin were found to be enriched in glomerular extracts (see Table 1, p. 1498). Antibodies to C1q may facilitate the formation of large immune complexes by binding to solid-phase bounde C1q. The enrichment of antibodies to Sm, SSA(Ro) and SSB(La) exceeded the frequency of antibodies to dsDNA. This is the first demonstration of enrichment of antibodies to Sm in glomerular immune deposits of SLE. The observed binding to histone was due to aggregated IgG, not to this monomeric analyte specificity. In one sample, anti-myeloperoxidase antibodies was enriched. Antibodies to cathepsin G, lactoferrin and ß2-glycoprotein I were not detected, although 5 of the 23 kidney specimens studied were obtained from patients who died from the antiphospholipid syndrome. However, the identified IgG only amounted to a small percentage (below 1 %) of total recovered IgG so that the bulk of IgG in the extracts remains unexplained.

Anyway, the notable finding of this study is that autoantibodies with multiple specificities were found to be enriched in the glomerular immune deposits of SLE patients. This may have therapeutic implications.

September 09/03:

Scl70 (Anti-DNA Topoisomerase I) Antibodies: Correlation of Titer with Disease Activity

Systemic sclerosis (scleroderma) is a generalized disease of connective tissue that is characterized by fibrosis and vascular obliteration affecting the skin and certain internal organs, including the lungs, heart, gastrointestinal tract and kidneys. There are two different forms of scleroderma: a diffuse variant with wide-spread cutaneous thickening that is associated with anti-Scl 70 (anti-DNA topoisomerase I) antibodies and a limited variant with cutaneous thickening restricted to hands, face and neck that is associated with anti-CENP-B antibodies.

In contrast to anti-dsDNA antibodies that display a clear correlation with disease activity, studies attempting to find such an association for antibodies directed against ENAs (extractable nuclear antigens) led to conflicting results.

The presented recent publication addresses this important question for anti-Scl70 antibodies in systemic sclerosis:

Hu PQ, Fertig N, Medsger TA, Wright TM (2003)
Correlation of Serum Anti-DNA Topoisomerase I Antibody Levels With Disease Severity and Activity in Systemic Sclerosis
Arthritis Rheum 48, 1363 – 1373

59 patients with diffuse systemic sclerosis were analyzed for disease activity and additionally for disease severity using the total skin score (TSS) that grades skin thickness. Furthermore, anti-Scl70 (DNA-topoisomerase I) antibodies of the isotypes IgG and IgA and all IgG subclasses were determined by in-house assays. From 11 of these 59 patients, serial serum samples were obtained.

The anti-Scl70 titer for IgG antibodies was highly correlated with disease severity (see Figure 3) and disease activity (see Figure 4). Patients with no or mild skin thickening had a mean titer of 54.6 units, whereas patients with moderate and severe/end-stage thickening had titers of 141.7 and 155.2 units, respectively. For disease activity, the mean anti-Scl70 titers were 135.16 units, 81.68 units and 59.9 units in patients with very active, mildly/moderately active and inactive disease, respectively. Parallel changes of the TSS and levels of IgG and IgA anti-Scl70 antibodies over time were obvious from the analysis of serial samples (see Figure 5). These results provide evidence for a positive correlation of anti-Scl70 antibody levels with disease activity and disease severity in systemic sclerosis.

August 08/03:

ANCA: Pathogenesis

Antineutrophil cytoplasmic antibodies (ANCA) directed against the neutrophil antigens PR3 and MPO are a feature of ANCA-associated small vessel vasculitides (ASSV): Wegener's granulomatosis, microscopic polyangiitis and Churg-Strauss syndrome.

Although these autoantibodies are considered as being pathogenic by many authors, the underlying mechanisms are far from being resolved. One of the most widely accepted models is the following: in 1990, it was first described that ANCA induce neutrophils to degranulate and produce oxygen radicals in vitro. Later it was shown that they also release granule proteins, cytokines, chemokines, and adhesion molecules. These effects were shown to be markedly enhanced by priming the neutrophils with cytokines such as tumor necrosis factor. Priming leads to the expression of small amounts of granule constituents (including ANCA target antigens) at the cell surface where they are accessible to interact with ANCA. Leukocytes such as granulocytes that have been activated by ANCA may adhere to endothelium and cause endothelial cell damage thereby leading to vasculitis.

More details with respect to currently discussed pathogenetic mechanisms are found in the following reviews:

Falk RJ, Jennette JC (2002)
ANCA are Pathogenic - Oh Yes They Are !
J Am Soc Nephrol 13, 1977 - 1979

Hewins P, Savage C (2003)
Anti-neutrophil cytoplasm antibody associated vasculitis
Int J Biochem Cell Biol 35, 277 - 282

The signal mechanisms for activation of neutrophils rely both on the Fc gamma and on the F(ab')2 part of the ANCA molecule (see page 279 in the second article). Additionally, ß2integrins that are known to be relevant for cell adhesion processes, are involved in the activation process of neutrophils.

Although increasing support for a pathogenic role of ANCA comes from animal studies (see first publication), it should be kept in mind that cell-mediated processes may be of equal importance.

July 07/03:

Celiac Disease: excellent concordance of Celikey with IIF (endomysial antibodies)

Celiac disease is an autoimmune disorder induced in genetically susceptible individuals by the ingestion of cereals, such as rye, barley or wheat. About twenty years ago, mainly the classical form of the disease was diagnosed that is characterized by malabsorption and failure to thrive in children. During the past two decades, however, the clinical picture has changed to include milder forms.

Induced by the development and application of serological screening methods, it became obvious that celiac disease is one of the most common lifelong disorders in both Europe and the United States. Serological screening methods mainly include indirect immunofluorescence (IIF) using monkey oesophagus (endomysial antibodies) and as a more recent development ELISAs with tissue transglutaminase as antigen. Celikey that employs recombinant human tissue transglutaminase from the baculovirus expression system proved to be one of the best ELISAs in various former studies (see PoM July 2002 07/02).

In the following investigation, an extensive screening of Finnish students was performed to test the comparability of Celikey with IIF (endomysial antibodies) and to estimate the prevalence of celiac disease among Finnish children:

Mäki M, Mustalahti K, Kokkonen J, et al. (2003)
Prevalence of Celiac Disease among Children in Finland
N Engl J Med 348, 2517 - 2524

Sera from 3,654 students (age range, 7 to 16 years) were screened for endomysial and tissue transglutaminase antibodies. HLA-typing was also performed. Of the 3,654 subjects, 56 (1.5 %) had positive antibody tests. The results of the two screening methods were highly concordant (only 3 discrepant results!). None of the 3,654 subjects had shown symptoms of celiac disease before collection of sera but 10 of those with positive antibody tests developed this disorder during the period between sera collection and testing (7 years). Of the remaining 36 subjects who agreed to undergo biopsy, 27 had evidence of celiac disease on biopsy. All but two of these antibody-positive subjects had one of the disease-related HLA-DQ2 or HLA-DQ8 haplotypes. Based on this study, the prevalence of celiac disease among Finnish schoolchildren is at least one case in 99.

June 06/03:

Measurement of ANCA Titers as Tool for Prediction of Relapses in Vasculitides

ANCA (antineutrophil cytoplasmic antibodies) are associated with a spectrum of vasculitides that includes Wegener's granulomatosis, microscopic polyangiitis, Churg Strauss syndrome and primary pauci-immune necrotizing and crescentic glomerulonephritis. Only two types of ANCA, antiproteinase 3 (PR3) and antimyeloperoxi-dase (MPO) antibodies account for this association.

Although the diagnostic value of PR3- and MPO-ANCA tests is well established, the usefulness of determining ANCA titers for the assessment of disease activity is discussed controversially. Absolute titers correlate only roughly with disease severity. For PR3-ANCA, however, it has been reported that rising titers during remission may precede relapses in patients with Wegener's granulomatosis. For MPO-ANCA, only limited data with respect to this item are available.

In the following recent publication, additional, significant data with respect to the utility of monitoring PR3- and MPO-ANCA titers for the prediction of relapses and of preemptive enhancement of immunosuppression for prevention of relapses are presented:

Han WK, Choi HK, Roth RM, et al. (2003)
Serial ANCA titers: Useful tool for prevention of relapses in ANCA-associated vasculitis
Kidney Int 63, 1079 - 1085

The study included 48 patients with PR3- or MPO-ANCA and with characteristic clinical and/or histological features of Wegener's granulomatosis or microscopic polyangiitis, as defined by the Chapel Hill consensus conference, or with clinical evidence of rapidly progressive glomerulonephritis. They were followed up for a mean of 46.2 months, with ANCA titers determined at intervals of 2.3 months (on average).

In total, 23 disease relapses were observed in 16 patients. Of these 23 relapses, 12 were preceded by an at least fourfold rise in either MPO- or PR3-ANCA titer. Two other relapses were associated with a concomitant rise in ANCA titer of at least fourfold, 3 were preceded by titer rises of less than fourfold and the remaining 6 relapses were accompanied by negative or falling titers of ANCA. 5 of these 6 relapses, however, were confined to nose, sinuses and/or mouth. Evidently, in 74 % of all observed relapses, a rise in ANCA titer was detected. In 52 % of all cases an at least fourfold rise in ANCA titer preceded the relapse.

In order to assess the usefulness of preemptive enhancement of immunosuppression, the patients were divided into two groups. 8 patients with 10 at least fourfold rises in ANCA titer did not receive increased treatment at the time of the titer change. 11 patients, each with one titer rise of at least fourfold, on the other hand were given enhanced immunosuppressive medications preemptively after the occurrence of the titer rise. Among the first group, all patients had relapses after each titer rise that occurred between 2 and 12 months later, whereas in the second group only 2 relapses were seen, at 3 and at 6 months after the titer change.

The present study provides evidence in support of the view that strong rises in ANCA titer during remission might predict disease relapses and that serial measurements of

ANCA titer during remission are of value for the management of patients (preemptive enhancement of immunosuppression).

As further studies with respect to the value of monitoring ANCA for prediction of disease relapses are required, it might be interesting and worthwhile to initiate such studies in the Market Companies using EliA MPO- and PR3-ANCA.

May 05/03:

IIF on Hep-2 and Hep-2000 cells does not detect all ENA specificities

Currently, indirect immunofluorescence (IIF) is still the traditional and most widely accepted method for ANA screening. In the past, animal substrates such as mouse kidney/liver or rat liver have been used, but today human epithelioid cells (Hep-2 cells, a human larynx epithelioma cell line) are applied most frequently, due to increased sensitivity. Particularly the Hep-2000 substrate, that consists of HEp-2 cells transfected with SSA/Ro cDNA, is regarded as valuable tool because it has a higher sensitivity for detecting SSA/Ro.

IIF on Hep-2/Hep-2000 cells usually is part of a cascade testing approach, which means testing for ENA specificities by other methods is only performed if the initial screening test is positive. In some countries, reimbursement for further ENA assays is provided only for samples with positive IIF.

As it has been suggested that some ENAs, such as anti-SSA/Ro or anti-SSB/La antibodies, may be missed on IIF, the authors of the following recent publication have performed a prospective study designed to evaluate the sensitivity of IIF for detection of ENAs:

Hoffman IE, Peene I, Veys EM, De Keyser F (2002)
Detection of Specific Antinuclear Reactivities in Patients with Negative Anti-nuclear Antibody Immunofluorescence Screening Tests
Clin Chem 48, 2171 - 2176

494 Serum samples were included in the study. All samples were collected prospectively for routine ANA testing from August to December 2000 at the Department of Rheumatology, Ghent University Hospital. All sera were analyzed by IIF on Hep-2 and Hep-2000 cells and by LIA (INNO-LIA ANA Update, Innogenetics). IIF-negative samples with reactivity in LIA were additionally tested with a commercially available ELISA (Quanta-lite, Inova Diagnostics) for the corresponding specificity and by double immunodiffusion in Ouchterlony plates with thymus/spleen nuclear extract (Immunoconcepts).

The authors found a good correlation between the fluorescence intensity and patterns of both IIF substrates. The percentages of samples being tested positive by LIA are depicted according to IIF intensity in Figure 1 (p. 2173). It is evident that 12/291 (4.1 %) of samples tested negative by IIF displayed reactivity in the LIA. Four samples showed discrepant IIF results and were positive by LIA. The results for these 16 sera are shown in Table 3 (p. 2174). 7 of these sera showed SSB/La reactivity in LIA that was partly confirmed by ELISA (1 positive, 3 borderline). The other reactivities observed were SSA/Ro (4), Jo1 (2), histones (2), RNP (4), Scl70 (1) and Sm D (1). There were several patient sera with multiple specificities. One case of Jo1 positivity and one case of SSA/Ro reactivity were confirmed by ELISA. As double immunodiffusion is known to be less sensitive than LIA, it is to be expected that only few LIA results were confirmed by this method.

Some of these patients had defined rheumatic diseases such as rheumatoid arthritis (patient 7), SLE (patient 4), Sjögren syndrome (patient 13) and polymyositis (patient 5).

Others had symptoms suggestive of connective tissue disease (patients 6, 11 and 12), although no definite diagnosis could be established.

The authors conclude from these findings that whenever clinical suspicion for rheumatic disease is given, testing for ENAs should be performed, even in case of negative IIF results. Otherwise there would be a risk of missing anti-SSA/Ro, anti-SSB/La and anti-Jo1 antibodies.

April 04/03:

Antineutrophil Cytoplasmic Antibodies

Antineutrophil cytoplasmic antibodies (ANCA) were first described in 1982 in a few patients with segmental necrotizing glomerulonephritis. Already in 1985, C-ANCA were recognized as sensitive and specific marker of Wegener's granulomatosis. Until 1990, ANCA were detected exclusively by indirect immunofluorescence on ethanol-fixed neutrophils as substrate. C-ANCA produce a cytoplasmic fluorescence pattern whereas P-ANCA lead to a perinuclear to nuclear staining. Around 1990, PR3 was identified as antigen for C-ANCA and MPO was assigned as main antigen of P-ANCA. Since then, both antigens were purified and used for the development of ELISAs. With respect to the application of both IIF and ELISA, in 1999 an International Consensus Statement was published (see PoM July 7/99). It was recommended to screen for ANCA by IIF and to use ELISA for confirmation of positive results. On the other hand, the significance of both methods is still being discussed controversially. There are also approaches to employ ELISA as screening test for ANCA with subsequent confirmation of positive results by IIF (see PoM February 02/00, see PoM November 11/02).

The main disadvantage of IIF compared to ELISA is that it is an elaborate and subjective technique, in contrast to ELISA that is easy-to-perform and leads to quantifyable, objective results. The following publication shows that variations in the IIF patterns for individual test sera as revealed by external quality assessment schemes are not in any case caused by errors in the interpretation but may also be due to technical factors:

Pollock W, Clarke K, Gallagher K, et al. (2002)
Immunofluorescent patterns produced by Antineutrophil cytoplasmic antibodies (ANCA) vary depending on neutrophil substrate and conjugate
J Clin Pathol 55, 680 - 683

Two sera with C-ANCA and PR3 specificity and two sera with P-ANCA, one with MPO specificity, were distributed to 11 laboratories and tested with the neutrophil substrate and the entire technical procedure routinely used in that laboratory. All descriptions of IIF patterns were confirmed on retesting in individual laboratories and on review by the Australian ANCA study group thereby minimizing interpretation errors. The four sera were positive for ANCA by IIF in all laboratories. However, 8 out of 44 results were inconsistent with the consensus patterns. One was an atypical C-ANCA pattern (occurring in rheumatoid arthritis or inflammatory bowel disease) instead of a classical C-ANCA fluorescence. The other 7 resulted in at least some cytoplasmic fluorescence when the consensus pattern was P-ANCA (4 with MPO specificity, 3 without). In one case, only cytoplasmic fluorescence was present. These results reveal that technical differences might also be a reason for variations in IIF patterns.

March 03/03:

Celiac Disease

Celiac disease (CD) is a genetically based autoimmune disease going along with permanent intolerance to gluten (that is contained in cereals like rye or barley). It is characterized by a flattening of the intestinal mucosa and malabsorption. Recently, serological methods of detecting antibodies to gliadin, endomysium (detected by indirect immunofluorescence) or tissue transglutaminase (tTG) have become preferred tools of diagnosing both symptomatic and asymptomatic patients with CD. Particularly, tTG antibody assays have obtained a huge relevance, due to their high sensitivity and specificity.

Tissue transglutaminase or type 2 transglutaminase belongs to a family of seven isoforms of enzymes involved in protein crosslinking. tTG is a Ca^{++} dependent ubiquitous intracellular enzyme that catalyzes the covalent formation of ?-lysine bonds. The human enzyme consists of four domains.

The scope of the presented publication was to map the antigenic region of human tTG:

Sblattero D, Florian F, Azzoni E, et al. (2002)
The analysis of the fine specificity of celiac disease antibodies using tissue transglutaminase fragments
Eur J Biochem 269, 5175 - 5181

The authors had identified tTG antibodies from phage display libraries created from intestinal lymphocytes from CD patients before. In this publication, they have used these antibodies to localize the antigenic recognition site of tTG. They identified a conformational region in the core domain that is also recognized by patients' sera. Indirect hints at a conformational nature of the binding site had already come from the fact that the performance of Celikey (that contains baculovirus-expressed antigen) was repeatedly found to be superiour to other assays tested.

Celiac disease is diagnosed in the general population with a prevalence of 0.5% to 1%. As it has been reported to be massively underdiagnosed due to a high frequency of silent or atypical forms, general population screening for this disorder is a matter of debate.

This topic is discussed in the following articles: the protagonist article by A. Fasano and the antagonist article by P.J. Kumar.

Fasano A (2003)
Protagonist. European and North American populations should be screened for celiac disease
Gut 52, 168 - 169

Kumar PJ (2003)
Antagonist. European and North American populations should be screened for celiac disease
Gut 52, 170 - 171

According to A. Fasano, general population screening for CD fulfills all five WHO criteria for mass screening:

1. Early clinical detection is often difficult due to silent or atypical forms.

2. The prevalence of the disease is high. In areas where CD has been historically considered rare (mainly USA), its prevalence has been shown to be similar to that reported for Europe (*Fasano A, Berti I, Gerarduzzi T* et al. **Prevalence of Celiac Disease in At-Risk and Not-at-Risk Groups in the United States**, Arch Intern Med 163, 286 - 292).

3. The detection of tTG as autoantigen has rendered possible the development of sensitive, specific and easy-to-perform screening tests.

4. Effective treatment for CD is available (strict gluten-free diet)

5. Patients with undiagnosed and untreated CD as well as those diagnosed later in life, have an increased morbidity and mortality risk due to associated disorders. This can be prevented by early diagnosis and treatment.

As long as detailed cost effective analyses are missing, Fasano suggests a systematic process for case finding, in which patients with symptoms and/or conditions known to be associated with CD are targeted.

In contrast, P.J. Kumar in the antagonist article denies an effect of general population screening on morbidity and mortality of CD and assumes that asymptomatic, screen detected patients would show poor compliance with a gluten-free diet (a study from Italy).

February 02/03:

Liver Kidney Microsomal type 1 (LKM 1) Antibodies

Liver kidney microsomal antibody type 1 (LKM1) is the diagnostic marker of autoimmune hepatitis type 2 (AIH2) that typically affects children and young adults. As AIH2 often presents as acute hepatitis or even with fulminant hepatic failure, serological analysis plays an important role for diagnosis. LKM1 is conventionally detected by indirect immunofluorescence (IIF), using rat liver, kidney and stomach as composite substrate. However, employing this technique, LKM1 is often misidentified as it shows similar patterns to antimitochondrial antibodies and liver cytosol type 1 antibodies. Therefore alternate techniques would be of advantage. Since the relevant antigen was identified as cytochrome P4502D6 in 1988, ELISAs were developed, first for research use and then as commercial assays.

In the following recent publication two commercial assays employing recombinant antigens (Varelisa from Pharmacia using antigen expressed in the baculovirus/insect cell system and LKM1-ELISA from MBL using procaryotically expressed antigen) were assayed for their diagnostic value by comparison with indirect immunofluorescence, in house ELISA and a radioligand assay:

Kerkar N, Ma Y, Davies ET, et al. (2002)
Detection of liver kidney microsomal type 1 antibody using molecularly based immunoassays
J Clin Pathol 55, 906 - 909

30 Sera, taken at different stages of disease activity from three children with AIH2 symptoms and reflecting different titres were investigated. Sera from 45 LKM1 negative patients were also tested. As normal controls, sera from 10 healthy children were studied.

All 30 sera of the patients with AIH2 and none of the control sera were positive by IIF, radioligand assay and in house ELISA. Using Varelisa, one of the LKM1 positive sera was measured negative and one of the control sera was detected as positive. Interestingly, the only serum from the LKM1 negative group that was positive by Varelisa came from a patient with AIH1 who 5 years previously was weakly positive for LKM1 as detected by IIF. All 30 sera from the patient group were positive employing the LKM1-ELISA from MBL. However, 14 sera of the control groups were also measured positive. After adjustment of the Cut-off for the LKM1-ELISA from MBL, all the sera from the LKM1 positive patients but only 2 from the control groups remained positive.

The authors conclude that the Varelisa kit allows accurate detection of LKM1 whereas a more clinically relevant cut-off point needs to be established before the LKM1-ELISA from MBL can be of diagnostic use.

January 01/03:
Antiphospholipid Syndrome

More than 15 years ago Harris, Hughes and others have described the features of the antiphospholipid syndrome (APS). It includes the presence of antiphospholipid antibodies and is associated with clinical manifestations such as arterial and venous thrombosis, recurrent abortions and thrombocytopenia. Although being more prevalent in patients with systemic lupus erythematosus as "secondary APS", antiphospholipid antibodies also occur in patients without other manifestations of autoimmune disease ("primary APS"). Recently, renal manifestations of the APS have received more attention as the kidney appears to be a major target organ in both primary and secondary APS.

More details concerning pathogenesis of APS, renal syndromes in primary and secondary APS, antiphospholipid antibodies in further conditions (systemic hypertension, end-stage renal disease, renal transplantation) and management of thrombosis in APS are found in the following recent publication:

Nzerue CM, Hewan-Lowe K , Pierangeli S, Harris EN (2002)
"Black Swan in the kidney": Renal involvement in the antiphospholipid antibody syndrome
Kidney Int 62, 733 - 744

Antiphospholipid antibodies include lupus anticoagulant and anticardiolipin antibodies. Lupus anticoagulant is detected in coagulation assays as it slows the rate of thrombin generation by perturbing interactions that require phospholipid, thus acting as an anticoagulant. Anticardiolipin antibodies, in contrast, are recognized by the ability to bind anionic phospholipids in solid-phase immunoassays. In vivo, both are thought to induce thromboses. Induction of thrombosis by antiphospholipid antibodies involves interference with endogenous anticoagulant systems (such as protein C or annexin V), cellular activation (involving platelets, endothelial cells and leukocytes), persistent activation of the coagulation system (due to tissue factor activation and inhibition of the antithrombin III pathway), as well as inhibition of fibrinolysis. For details with respect to coagulation see Figure 1, page 735.

The kidney manifestations of APS most probably result from thrombosis occurring at any location within the renal vasculature. In case of secondary APS, it has been reported that the presence of antiphospholipid antibodies consistently correlates with the occurrence of renal thrombotic microangiopathy which might be associated with worse prognosis. In contrast to the well-described nephropathy associated with SLE and APS, it was not widely appreciated that the primary APS could lead to nephropathy until recently. The renal lesions of the primary APS are identical to those seen in other thrombotic microangiopathies and several patients present with acute renal failure.

For treatment of APS associated thrombosis, anticoagulation is indicated. As the risk of recurrence is very high, lifelong therapy is favored. Plasmapheresis may be beneficial in APS patients presenting with thrombotic microangiopathy and should be definitely considered in patients with catastrophic APS. The role of chloroquine and hydroxychloroquine, respectively, is discussed controversially. Although it has been shown that thrombus size and total time of thrombus formation were reduced in mice, the effect on APS in humans is unclear. Experience with intravenous immunoglobulins in treating APS is limited and uncontrolled.

Recurrent miscarriage is one of the major symptoms of APS. As the pathogenesis of APS-associated pregnancy loss is assumed to be linked to placental thrombosis, many centers have adopted a thromboprophylactic treatment in the form of low-dose aspirin and/or heparin. Clear consensus with respect to optimal therapy in practice, however, is absent as the superiority of the combination of low-dose aspirin plus heparin compared to low-dose aspirin alone is discussed controversially.

In the following publication, a randomized, controlled trial is presented that addresses this open question:

Farquharson RG, Quenby S, Greaves M (2002)
Antiphospholipid Syndrome in Pregnancy: A Randomized, Controlled Trial of Treatment
Obstet Gynecol 100, 408 - 413

The aim of the trial was to assess whether the combination of low-dose aspirin plus low molecular weight heparin was superior to low-dose aspirin alone in the prevention of miscarriage in a group of women with a history of recurrent pregnancy loss and positive diagnostic tests for APS. 98 randomized women were included in the trial. 47 of them received low-dose aspirin and 51 received low-dose aspirin plus low molecular weight heparin. The live-birth rate was 72 % in the first group and 78 % in the second group. Evidently, a high success rate was achieved when low-dose aspirin was applied alone and the addition of low molecular weight heparin did not significantly improve pregnancy outcome.