VEGF and diabetic microvascular complications

Sir,

Microvascular complications of diabetes mellitus contribute to the morbidity and mortality associated with the disease. The mediators of microvascular complications such as diabetic retinopathy and nephropathy continue to be subject of research and speculation. Growth factors have been implicated [1]. Recently, interest has focused on vascular endothelial growth factor (VEGF) in view of its role in tissue angiogenesis and its relevance to the development of diabetic retinopathy [2,3]. In this study, we have studied changes in circulating levels of VEGF in a group of diabetic patients with and without microvascular complications (retinopathy and nephropathy).

We studied 76 diabetic patients (20 type I and 56 type II). Their age was 49 ± 12 years (M ± SD) and the duration of known diabetes was 91 ± 71 months. Of these, 34 patients (44.7%) had some degree of diabetic retinopathy and 48 patients (54%) had some degree of renal involvement (41 patients had microalbuminuria and 7 had macroalbuminuria). Renal function (creatinine clearance) was within the normal range for the majority (129 ± 17 ml/min). Vascular endothelial growth factor (VEGF) was measured in all the patients by a radioimmunoassay (R & D Systems, UK). Comparison between the groups was undertaken by one way analysis of variance (ANOVA). Patients were compared to a group of 28 age-matched controls. A P value of < 0.05 was considered significant.

We noted a marked elevation of serum VEGF in diabetic patients compared to normal controls: DM: 421 ± 309 pg/ml and controls: 188 ± 145 pg/ml, P < 0.05. When we analysed different subgroups of diabetic patients, we noted that those with proliferative retinopathy had the highest levels (724 ± 376 pg/ml). Notably, patients with background retinopathy had circulating VEGF levels (379 ± 250 pg/ml) comparable to those of diabetic patients without retinopathy and normal individuals. Similarly, patients with microalbuminuria did not display elevated levels of VEGF. In contrast, patients with more advanced diabetic nephropathy and macroalbuminuria had significantly higher levels (662 ± 276 pg/ml; P < 0.05 compared to those with microalbuminuria). We also noted a weak but significant correlation between the level of albuminuria and serum VEGF (r = 0.27, P < 0.05). A VEGF value of 450 pg/ml discriminated between patients with and without nephropathy (micro- and macroalbuminuria) showing a sensitivity of 71% and a specificity of 50%.

In conclusion, we observed an elevation of circulating VEGF levels in diabetic patients with advanced microvascular complications such as proliferative retinopathy and macroalbuminuria. We can only speculate on the relevance of these observations to the pathogenesis of these complications. As far as diabetic proliferative retinopathy is concerned, a role for VEGF in retinal angiogenesis has been suggested and reinforced by observations of high vitreous levels of this growth factor in patients with this complication [3].

Regarding diabetic nephropathy, VEGF is also known as vascular permeability factor and has therefore been implicated in the pathogenesis of proteinuria [4]. Its elevation in diabetic patients with macroalbuminuria may suggest a causal association. Vascular endothelial growth factor has been shown to be upregulated in the renal tubular cells of patients with chronically ischaemic and hypoxic kidneys due to narrowing of preglomerular vessels [5]. Similar vascular changes have been described in diabetic kidneys [6], suggesting that these kidneys may also be the source of VEGF.

Further research is warranted to define the nature of the association between VEGF and the development of diabetic microvascular complications.

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Exacerbation of microscopic polyarteritis with azathioprine

Sir,

We present a case of probable azathioprine-associated exacerbation of microscopic polyarteritis.

A 19-year-old male presented in 1988 with nausea, malaise, hypertension, haematuria, proteinuria, fleeting polyarthritis and vasculitic rash following an episode of tonsillitis. Plasma creatinine was 0.11 mmol/l. Renal biopsy showed a focal segmental glomerulonephritis. A good response was achieved with pulse methylprednisolone followed by oral prednisolone. Tapering of prednisolone was attempted on several occasions with recurrence of the rash and episodes of pleuritic pain. Creatinine remained stable at 0.10 mmol/l with normal protein excretion and minimal haematuria. pANCA titre fluctu-
ated from $1:160$ to $1:640$. Skin biopsy revealed a neutrophilic vasculitis with dural abscess formation. Repeat renal biopsy in March 1995 revealed focal global sclerosis without vasculitis.

In May 1995, he was still requiring 25 mg prednisolone daily and had developed corticosteroid side-effects including obesity, striae and hypertension. Azathioprine at 100 mg daily was introduced and prednisolone was reduced to 22.5 mg daily. One month later he suffered high fevers, severe constitutional symptoms, pancreatitis (nausea, vomiting, abdominal pain and raised serum amylase) and rash. Widespread purpuric papules and nodules were present on the limbs and face, some with necrosis. There was no oral or nail involvement. Cryoglobulins were negative.

Skin biopsy revealed a heavy dermal neutrophilic infiltrate with vasculitis of small dermal vessels. Prednisolone was increased to 75 mg daily and azathioprine was changed to cyclophosphamide 100 mg daily. The rash settled within 2 weeks. Over the next 5 months prednisolone was reduced to 25 mg daily. He remained well, apart from one episode of pleuritic pain and another episode of mild rash, which settled without an increase in steroid therapy. pANCA was not elevated by ELISA.

In December 1995, there was concern regarding the continuing cyclophosphamide and higher dosage of prednisolone. It was uncertain whether azathioprine was responsible for the previous episode of vasculitis. Prednisolone was reduced to 20 mg daily and cyclophosphamide was changed to azathioprine 175 mg daily. One week later he presented with nausea, arthralgias and rash. He was afebrile. Tender erythematous nodules were present on the lower legs with underlying petechiae and capillaritis. Older violaceous plaques were present on the lower abdomen and upper limbs. Skin biopsy revealed a subacute small vessel vasculitis. Azathioprine was ceased and cyclophosphamide at 100 mg daily was recommenced. He again improved within 2 weeks. In January 1996 cyclophosphamide was replaced with cyclosporine 200 mg twice daily. He has remained well with negative pANCA.

Azathioprine has been associated with rare hypersensitivity reactions including fever, hypotension, leukocytosis, pancreatitis, hepatitis and acute interstitial nephritis. Bergman et al. [1] reported two cases of hypersensitivity vasculitis in haemodialysis patients receiving azathioprine for pre-transplant blood transfusions, one after 2 weeks, the other after 8 days. Both were confirmed with reactions within 6 hours of rechallenge. Beckett et al. [2] reported a case of leukocytoclastic vasculitis at 9 days after commencement of azathioprine in a patient with immune thrombocytopenia and a monoclonal gammopathy. Initially febrile, the patient became afebrile 24 hours after ceasing azathioprine. Immediate rechallenge was associated with prompt recurrence of fever and appearance of a vasculitic skin rash.

Azathioprine is commonly used in the management of vasculitis. In our patient the symptoms and signs associated with microscopic polyarteritis were similar to those produced by azathioprine hypersensitivity. However, the reduction in prednisone from 25 mg to 22.5 mg daily, which preceded the first relapse, would appear insufficient by itself to produce disease relapse, especially in the presence of azathioprine-induced immunosuppression. Additionally, pancreatitis is rarely recorded with microscopic polyarteritis but can be caused by azathioprine.

During the second relapse three therapeutic changes were made; a reduction in prednisone from 25 mg daily to 20 mg daily, cessation of cyclophosphamide and introduction of azathioprine. Disease recurrence would be an unlikely cause for this relapse as, firstly, the reduction in prednisone was slight and, secondly, the effects of cyclophosphamide-induced immunosuppression should still be evident at 7 days.

Thus, in both relapses we suggest an active exacerbation of microscopic polyarteritis with azathioprine. The mechanism for this exacerbation is unclear but would include a drug-induced vasculitis. Hypersensitivity reactions to azathioprine should be considered in the differential diagnosis of recurrent symptoms in vasculitis patients. Alternatively, exacerbation may reflect the fact that azathioprine is a less potent immunosuppressive agent.

#### Proteinuria in healthy individuals over 75 years of age

Sir,

In ageing of the kidneys there is a reduction in the number of healthy, active nephrons [1,2]. This physiological evolution is balanced by hyperplasia and secondary hyperfiltration of the remaining healthy nephrons [3,4]. This hyperfiltration, although produced by a counterbalancing mechanism, may produce proteinuria due to the change in the haemodynamic balance of the healthy nephrons and to an increase in intraglomerular pressure [5-7].

To evaluate this situation we examined, in a prospective study, the frequency of appearance of proteinuria in healthy individuals over 75 years old and its relationship with clinical and laboratory findings.

We studied a total of 108 healthy individuals over 75 years old (mean age 82.6 ± 6.2 years, with a range of 75–92). Of these subjects, 58 were male and 50 female. All subjects were either hospitalized at our hospital or were examined at the outpatient facilities for problems other than nephrological, e.g. orthopaedic, ORL, or ophthalmological problems.

In all cases, before putting in a catheter or starting treatment with antibiotics, we took a sample of urine, under sterile conditions and discarding the urine at the beginning of urination. All samples were examined as follows: (1) quantitative measurement of protein content (in mg/l) with an automatic analyser; (2) culture of the urine (with the results examined at 24 and 48 h); (3) microscopic examination of the centrifuged sediment. At the same time we measured serum creatinine and recorded the blood pressures (taken at least three times at different intervals).

Under the same conditions we measured the protein in samples of urine taken from a control group of 30 healthy volunteers (18 males and 12 females) with a mean age of 42.2 ± 11.6 years.

The rate of occurrence of proteinuria was 62% in the group under study (67 of 108) and 37% in the control group (11 of 30). The difference was statistically significant ($P < 0.01$). The quantitative measurement of the protein in the urine samples from the study group was $148.6 ± 143.5$ mg/l,

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and from the control group 35.7±4.19 mg/l (P<0.001). The high rate of standard deviation as compared to the mean rate, in both groups, is caused by the grouping of measurements, including samples with zero protein content.

In six cases with proteinuria greater than 1 g/l, further examination ((a) electrophoresis and immunoelectrophoresis of urine and plasma proteins, (b) myelogram) verified the existence of benign hyper-g-globulinaemia of the elders.

The frequency and the quantity of proteinuria found in the group under study was independent of other findings such as arterial hypertension or the existence of red or white blood cells in the centrifuged sediment of the urine samples. The presence of the cells was evaluated during the statistical analysis both as a ‘simple’ presence, and as to their absolute numbers per optical field. Accordingly we did not find a relationship between the presence of proteinuria (frequency and quantity) and positive cultures of the samples. On the other hand, we found that the frequency of proteinuria was greater in cases where the serum creatinine was ≤0.8 mg% as compared to the cases with creatinine ≥1.8 mg% (43 and 24 cases respectively). These two serum creatinine ranges were used to separate the study group into two subgroups according to their renal function.

From the results of this study we concluded that proteinuria is a common finding in otherwise healthy individuals over 75 years old. Also, we concluded that this proteinuria is not due to the presence of pathological findings in the centrifuged sediment of the urine (red and/or white blood cells) or to a positive urine culture. There was no statistical correlation between the presence of proteinuria and the presence or level of arterial hypertension.

The most probable mechanism for the proteinuria of the study group is sclerosis of the glomeruli of the remaining healthy, although hyperfiltrating, nephrons; this is part of the normal ageing process, which causes a reduction in the number of active nephrons [8].

Reinforcing this view is the finding of the study that the frequency of proteinuria is greater in individuals with low serum creatinine. This fact shows that in cases with increased serum creatinine the glomerulosclerosis has advanced to such a degree so as to limit the permeability of the membrane, even in the presence of hyperfiltration. According to older [2,9] and newer [3,5,10] studies, the progression of the renal illness which creates a reduction in the active renal mass causes a haemodynamic redistribution expressed by the hyperfiltration of the remaining nephrons and resulting in proteinuria [6]. According to the degree of glomerulosclerosis, we may see different amounts of proteinuria, but always less than that required to result in the nephrotic syndrome. However, it must be pointed out that although the presence of proteinuria in older individuals signifies the presence of renal hyperfiltration, a progressive reduction in this proteinuria must be considered a negative finding, as it reflects a progression of the glomerulosclerosis that blocks the permeability of the membrane towards the protein, even in the presence of hyperfiltration [8].

In conclusion, proteinuria in healthy, older individuals is a common finding and is caused by hyperfiltration of the remaining healthy and active nephrons.

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Goodpasture’s syndrome with normal renal function

Sir,

We read with interest the case of Goodpasture’s syndrome with normal renal function by Young Min et al. [1]. The patient presented with haemoptysis and positive anti-GBM antibody but with little to find in the urine initially. Lung biopsy showed linear deposition of IgG and C3 in the alveoli. Kidney biopsy was not done, but there was no doubt that the kidneys were affected. Several explanations were offered for the predominant pulmonary manifestations of this case including distinct subtype or earlier stage of the disease, different antigenic epitope on type IV collagen, and preferential involvement by different IgG subclasses. We suggest that in addition to these, some symptoms simply attract patient attention more than others. Haemoptysis is an alarming symptom that would call for immediate attention to seek medical care, as in the following patient who sought medical attention on three separate occasions over 10 years because of haemoptysis, two of these episodes were associated with Goodpasture’s syndrome.

The patient was a 19-year-old female when first seen on 30 December 1979. She was a cigarette smoker who developed dyspnoea on exertion in September, followed by haemoptysis 2 weeks later. A diagnosis of bronchitis was made and she was advised to stop smoking. Her symptoms progressed and she became very anaemic and hypoxaemic on admission in December. Haemoglobin was 5.5 g/dl. Scattered inspiratory crepitations were noted in her chest and diffuse pulmonary infiltrate was seen on X-ray. Transbronchial lung biopsy revealed intra-alveolar haemorrhage and haemosiderin-laden macrophages, but was inconclusive for alveolar basement membrane staining on immunofluorescence. Renal function was normal with a creatinine clearance of 104 ml/min. Proteinuria ranged from 0.5 to 0.7 g/day.

Renal biopsy was postponed because of an active urinary tract infection. Bone-marrow biopsy confirmed depleted iron stores. Blood was sent to Scripps Clinic in La Jolla, California for anti-GBM antibody. The patient was treated with bedrest, O2 inhalation, antibiotics, blood transfusion, and iron replacement with gradual improvement, and was discharged.

The blood sample was positive for anti-GBM antibody but the result did not come back until 22 February 1980. More importantly, it was filed without being brought to medical attention. Meanwhile the patient was apparently doing fine until 17 January 1985 when she presented, again, with haemoptysis, chest pain, and dyspnoea. She denied any exposure to chemicals or hydrocarbons, but she had continued to smoke. She had no urinary symptoms of note apart from 'pink urine' for 2 days in September of 1984. Physical examination was unremarkable. Haemoglobin was 11.7 g/dl. Sputum cytology was positive for haemosiderin-laden macrophages. Chest X-ray showed subsegment atelectasis of the right lung base. In view of the previous positive anti-GBM antibody in the blood, a percutaneous kidney biopsy was done, despite normal renal function, urinalysis, and lack of significant proteinuria. The histology was normal by light-microscopy, and no ultrastructural changes were noted on electron-microscopy. However, strong glomerular deposits of IgG and mild irregular deposits of C3 were found by immunofluorescence study. Prednisone was started at a dose of 60 mg/day, gradually tapered, and then stopped by 6 months. Intravenous cyclophosphamide was also given at 1 g every 6 weeks for four doses. She did not receive plasma exchange. Anti-GBM antibody was monitored monthly and remained positive until April of 1985, becoming negative thereafter. The patient was last seen in February of 1990 with blood-streaked sputum. She had been started on prednisone 10 days earlier by the family doctor. Haemoglobin was 13.6 g/dl. Chest was clear. Chest X-ray was normal. Urinalysis was unremarkable, and renal function remained normal. Anti-GBM antibody also remained negative, and the prednisone was stopped.

Like most patients with Goodpasture's syndrome, this patient had involvement of the lung and the kidney by anti-GBM disease from the start, but it was because of the pulmonary manifestations that she sought medical attention at the beginning and subsequently. By nature, Goodpasture's syndrome is a rapidly progressive disease and if untreated, most patients die. She presumably had anti-GBM antibody in the blood and the kidney for at least 5 years. It is remarkable that her renal disease did not progress to a more fulminant stage nor did she succumb to pulmonary haemorrhage or renal failure despite being withheld from treatment, inadvertently, over this period. At present, she is a mother of 3 and has had no haemoptysis in the last 6 years.

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Simultaneous relapse of minimal-change glomerulonephritis and Graves' disease

Sir,

We recently reported [1] the case of a patient who had a history of recurring steroid-dependent nephrotic syndrome resulting from minimal-change glomerulonephritis, which went into complete remission after cyclophosphamide therapy. Having achieved complete remission, the patient subsequently developed a relapse of the nephrotic syndrome and simultaneously (i) sarcoidosis and (ii) Graves' disease with thyroglobulin and thyroid microsomal antibodies.

The patient responded dramatically to treatment with methylprednisolone (administered because of sarcoidosis) and carbimazole treatment (administered because of thyrotoxicosis). At the time of writing [1] all signs of nephrotic syndrome, sarcoidosis and thyrotoxicosis had disappeared and the patient was completely asymptomatic after August 1995. ACE levels were in the normal range (41 U/l), but elevated thyroglobulin (1 : 638 400) and thyroid microsomal (TPO) antibody titres (1 : 409 600) persisted.

In May 1996, the patient had a relapse of the nephrotic syndrome (8.2 g protein/24 h) and two episodes of supraventricular tachycardia, reversible with carotid pressure and TSH concentration was subnormal. Proteinuria responded promptly (within 6 days) to 60 mg methylprednisolone p.o., but the patient developed fatigue, loose stools, sweating, and nervousness. He failed to visit the outpatient clinic because he feared losing his job, but when presenting in September 1996 he had signs of florid thyrotoxicosis (TSH 0.06 μU/ml; T3 4.4 ng/ml and T4 17.8 μg/dl) which promptly responded to carbimazole. Thoracic X-ray and ACE concentrations excluded a relapse of sarcoidosis.

The virtually simultaneous occurrence of nephrotic syndrome and thyrotoxicosis lend further credence to the hypothesis, proposed in the original report, that the two immunological abnormalities are linked by some unknown mechanism.

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Colchicine agranulocytosis

Sir,

Severe acute colchicine intoxication has been reported to occur when ordinarily non-toxic doses have been given to patients with renal disease [1]. We describe a patient with chronic renal failure who developed reversible agranulocytosis after short-term colchicine treatment at a very low dose.

A 86-year-old woman was admitted in our unit in August 1996 because of end-stage chronic renal failure of unknown origin. Anamnesis revealed no antecedent. She had received furosemide for 2 years. Physical examination showed right pleuritis and lower limb oedema. On admission significant laboratory data were as follows: serum creatinine, 670 μmol/l; haemoglobin, 85 g/l; leukocyte count, 9300/mm3 (neutrophils 67%); platelet count, 357 000/mm3; serum uric acid, 730 μmol/l.

Four days after admission, a peritoneal catheter was inserted and colchicine therapy (0.5 mg/day) was begun because of gouty arthritis. At the beginning of colchicine therapy, the leukocyte count was 8700/mm3. Seven days later, colchicine was stopped because of diarrhoea. The leukocyte count was 3500/mm3. Two days after colchicine withdrawal, polymorphonuclear neutrophils were less than 500/mm3. Bone-marrow aspiration was performed. Microscopical examination of the stained bone-marrow samples revealed increased cellularity with normal red-cell precursors; all stages of maturation of myeloid line were
represented with a shift to the left and toxic granulation. Agranulocytosis extended for 5 days. Serum colchicine concentration was 6 μg/l (normal range, 1–3 μg/l) 4 days after colchicine withdrawal.

No other cause of neutropenia than acute colchicine intoxication could be demonstrated in our patient. No other medication was prescribed. Self medication was excluded because of the patient’s disability. Infectious diseases (HBV, CMV, EBV) were eliminated by serological tests.

Our patient received colchicine for only 7 days and at a very reduced dosage. Nevertheless, agranulocytosis is likely to be related to colchicine. Indeed, microscopical examination of the stained bone-marrow samples was consistent with drug toxicity, agranulocytosis improved after colchicine withdrawal, and supratherapeutic serum colchicine concentration was found 4 days after drug withdrawal. Moreover no other cause of agranulocytosis could be demonstrated in our patient.

Increased toxicity of colchicine has been described in patients receiving concomitant therapy with P-450 enzyme inhibitors such as cimetidine, erythromycin, or tolbutamide [2]. Our patient received only frusemide in association with colchicine. Moreover no sign of hepatic dysfunction was present in our patient. The only risk factor for increased colchicine toxicity was severe renal dysfunction.

Clearance of the drug from plasma is reduced in renal failure [3], since 10% or more is excreted by the kidneys [4]. Severe acute colchicine intoxication has been reported to occur when ordinarily non-toxic doses have been given to patients with renal disease. In our patient, colchicine intoxication occurred despite very low dosage prescribed for a short period.

Our case report suggests that because of rapid accumulation and potential major side-effects, colchicine would be definitively proscribed in patients with severe renal insufficiency.

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A rare cause of acute tubulo-interstitial nephritis: Salmonella typhimurium infection

Sir,

Acute tubulo-interstitial nephropathy (TIN) is characterized by inflammation between the glomeruli in the areas surrounding the tubules, and tubular damage is generally present. Most common causes of TIN are drugs and infections [1,2]. Various infectious diseases associated with TIN have been reported, but the pathogenesis of infection related TIN is still unknown. Immune-mediated glomerulonephritis, pyelonephritis, or bacteremia during Salmonella typhimurium infections have been reported; however, salmonellosis leading to TIN is extremely rare [3–5].

We describe a childhood case with TIN secondary to Salmonella typhimurium infection.

A 12-year-old girl presented with a 15-day history of bloody diarrhoea, vomiting and intermittent macroscopic haematuria preceded with fever and headache. There was no history of drug ingestion. The past and family medical histories were unremarkable.

On physical examination the patient’s blood pressure was 110/70 mmHg, she was at the 25th percentile for weight and at the 50th percentile for height. Except for mild dehydration her physical examination was normal.

Her haemoglobin was 10.1 g/dl, haematocrit 31%, white blood cell count 5300/mm³ with a normal differential count. Erythrocyte morphology and clotting screen were also normal. Erythrocyte sedimentation rate was 66 mm/h. Urinalysis revealed a specific gravity of 1.008 with moderate proteinuria, and microscopy showed scanty red blood cells and coarse casts but no eosinophils. Urinary protein loss was 28 mg/m²/h. Serum sodium was 125 mEq/l, potassium 3.1 mEq/l, bicarbonate 19.3 mmol/l, blood urea nitrogen (BUN) 34 mg/dl, creatinine 2.1 mg/dl, and phosphorus 2.6.

Calcium, alkaline phosphatase, uric acid, total protein and albumin were all in normal ranges. Fractional excretion of sodium was 5.3% and tubular phosphate reabsorption was 68% (normal: >80%). Complement and serum immunoglobulins were normal. Abdominal ultrasonography revealed enlarged kidneys. The patient’s daily urine output was approximately 2 litres.

All bacteriological cultures were negative for Salmonella typhimurium, but an increase in O agglutinins was demonstrated (O agglutinin titre was negative on admission, and increased to 1:320 during the third week). The patient was treated with ciprofloxacin and her diarrhoea settled within 48 h of admission, whereas fever persisted for 5 days. Proteinuria and haematuria disappeared and renal function tests returned to normal within 8 days (BUN 12 mg/dl, creatinine 0.7 mg/dl).

The occurrence of self-limiting renal dysfunction often in the face of good urine output associated with various urinary abnormalities, such as hyposthenuria, proteinuria, haematuria, and granular casts during an infection should strongly suggest the diagnosis of acute TIN [6]. It is mostly associated with systemic infections such as streptococcal infections, toxoplasmosis, measles, brucellosis, infectious mononucleosis, and Mycoplasma pneumoniae.

Although it has been proposed that bacterial or viral antigens accumulate in the renal interstitium, leading to immune-complex formation or to the attraction of T lymphocytes in a delayed hypersensitivity reaction [7,8], there is little proof that immunological mechanisms are involved in infection-related acute TIN.

Interstitial nephritis has been reported in six patients with schistosomiasis and salmonellosis, and in one adult patient with Salmonella typhimurium infection [4,5]. In our patient, salmonellosis was diagnosed by the presence of bloody diarrhoea together with the demonstration of the increase in O agglutinins within 3 weeks. Self-limiting non-oliguric renal insufficiency along with hyposthenuria, moderate proteinuria, haematuria, presence of granular casts in the urine, and anaemia during Salmonella typhimurium infection is consistent with acute TIN. Eosinophilia, which was absent in this case, is expected in only half of the patients [6]. Pyelonephritis due to Salmonella typhimurium infection is excluded both with ultrasonographic findings and negative urine culture. Although the exact diagnosis is based on the
presence of the typical histopathological changes, renal biopsy was not performed in our patient bearing in mind the potential complications of kidney biopsy, and her renal function tests spontaneously improved within 8 days after the appropriate antimicrobial therapy had been initiated.

In conclusion, acute TIN must be considered in children as a cause of non-oliguric acute renal dysfunction during S. typhimurium infection. This case is an example of this rare association.


Another case of acute renal failure (ARF) due to acute tubular necrosis (ATN), proven by renal biopsy in non-fulminant hepatitis A virus (HAV) infection

Sir,

In addition to the excellent paper by Lin et al. in a recent issue of Nephrology Dialysis Transplantation we wish to report another case of ARF in non-fulminant HAV infection in whom renal biopsy was performed. This is still a very rare entity, with only 28 cases reported in the literature, without evidence for hepatorenal syndrome, other types of viral hepatitis, rhabdomyolysis, thrombotic microangiopathy, pregnancy, or severe hypotension [1–3].

A previously healthy 30-year-old man was admitted to the emergency department with a 4-day history of fever, malaise, vomiting, and decreased urine output. He had travelled to Spain 5 weeks before admission, where he had eaten raw (shell) fish. He had been taking non-steroidal anti-inflammatory drugs (NSAIDs) and antiemetics, prescribed by his general practitioner for “flu-like symptoms” for 2 days. He admitted to being a sporadic alcohol drinker and denied raw (shell) fish as well as contact with infected family members.

On examination the patient was sick and lethargic. His temperature was 40.3 °C. Respiratory rate was 28/min, the blood pressure was 124/74 mmHg, and the pulse was regular at 92 b.p.m. The patient was euvoeamic. Heart and lung auscultation were normal. There was mild right upper quadrant tenderness without guarding or rebound. Normal bowel sounds were present. Ascites was not detected. There was no hepatosplenomegaly nor enlarged lymph nodes. The sclerae were slightly icteric.

Initial laboratory tests disclosed the following values: SGOT/ASAT 8801 U/l, SGPT/ALAT 4625 U/l, LDH 18609 U/l, total bilirubin 2.9 mg/dl, alkaline phosphatase 154 U/l, γGT 278 U/l, lipase 257 U/l, CPK 546 U/l, creatinine 2.58 mg/dl, urea 55 mg/dl, uric acid 14.7 mg/dl, haemoglobin 11.9 g/dl, white blood cell count 9.1×10⁹/l with normal differential count, platelets 207×10⁹/l, prothrombin time 22% (INR 3.46), activated partial thromboplastin time 44.1 s (normal 24–38), D-dimers 7995 ng/ml (normal 0–500). Urinalysis demonstrated occult blood and 4.3 g/l protein. Urinary sodium was 65 mEq/l and urinary osmolality 304 mosm/l. Urinary sediment contained 4–6 RBC/h.p.f., 2–4 WBC/h.p.f., and sparse haemoglobin casts. The maximum values for liver and renal function impairment were: SGOT/ASAT 27300 U/l (day 2), SGPT/ALAT 13660 U/l (day 2), LDH 52500 U/l (day 2), total bilirubin 13.77 mg/dl (day 12), alkaline phosphatase 346 U/l (day 12), lipase 706 U/l (day 4), creatinine 20.2 mg/dl (day 10), urea 262 mg/dl (day 14), and uric acid 28.9 mg/dl (day 5).

Circulating immune complexes were negative. Complement and immunoglobulin levels were normal. Serology for HBV, HCV, HIV, EBV, and CMV was negative, but antihepatitis A IgM antibodies were positive. Toxicological screening was negative. Blood cultures drawn on admission remained sterile as well as urine cultures. Electrocardiogram, chest X-ray, and abdominal ultrasound were normal.

Haemodialysis was started on day 7. Because of persistent anuria renal biopsy was performed on day 15. Light-microscopy showed 14 glomeruli, none of which were obsolescent. The changes observed (focal tubular atrophy and tubular regeneration) were consistent with the recovery phase of ATN. Some tubules contained calcium oxalate crystals, whereas others contained bilirubin. Immunofluorescence tested negative with anti sera for C3, C1q, IgA, IgM, IgG, and fibrin. Electron microscopy was not done. Renal biopsy was complicated with a small AV fistula in the left kidney (seen on Doppler ultrasound) and transient haematuria. His clinical course was further remarkable except for a fever episode due to a cathether-related Staphylococcus aureus bacteraemia, successfully treated with flucloxacillin. Urine output recovered on day 22 and dialysis was stopped on day 26. Coagulation parameters normalized within 6 days, liver function tests within 3 weeks, and renal function after 6 weeks. The patient was discharged after 6 weeks, with a serum creatinine of 1.83 mg/dl and total bilirubin of 1.43 mg/dl.

In conclusion, ARF is a very rare complication of HAV infection with only 28 cases reported in the literature. We reviewed these 28 cases, and included the present case in the analysis. Most cases occurred in or after travelling to endemic areas, or when travelling in poor hygienic conditions. Eating raw (shell) fish as well as contact with infected family members (especially children with asymptomatic seroconversion) also seem to be risk factors. Renal biopsy was done in 16 of 29 cases (55%). Renal biopsy findings were normal in one (6.2%), and showed ATN in 11 (68.75%), acute interstitial nephritis in one (6.25%) and acute glomerulonephritis (mesangial proliferative) in three (18.75%). Immunofluorescence was positive only in four. Three of the 29 cases died, which gives a mortality of 10.34%. The exact pathophysiological mechanisms remain unsolved but are probably multifactorial, considering the heterogeneous findings on renal biopsy. Hepatorenal syndrome or functional renal failure are less likely in view of the high mean urinary sodium observed. In our patient the use of NSAIDs as well as the hyperuricaemia
and the elevated bilirubin might have contributed to the ARF, in view of the fact that some tubules filled with crystals and bilirubin were seen on renal biopsy. Other proposed mechanisms are cryoglobulinaemia or impaired reticuloendothelial function resulting in endotoxaemia and subsequent renal blood flow alterations [1–3]. The literature data are in favour of a hypothesis that the combination of either direct (virus-induced) or indirect (due to circulating immune complexes) renal injury and other contributing factors (hyperbilirubinaemia, hyperuricaemia, drugs) may eventually lead to ATN.

Total serum bilirubin and LDH, as reported previously, seem to be predictive for the need for dialysis [3]. As we stated earlier, the clinician should have a high clinical index of suspicion for HAV infection when treating patients presenting with ARF (e.g. after travelling to high-risk areas), since not all patients with ARF due to HAV infection present with icterus on admission (especially children) [2]. If possible renal biopsy should be performed in order to get a better understanding of the underlying pathophysiological mechanisms responsible for ARF in HAV infection, keeping in mind that even in the presence of normal coagulation parameters a substantially greater rate of complications after renal biopsy may occur (AV fistula, haematoma), as was also the case in our patient [2]. This may be due to uraemic thrombopathy. Finally, in an era with good HAV vaccination available, one should always advise travellers to undergo vaccination when travelling to endemic areas, in view of the possible complications, morbidity and mortality.

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Peritonitis by Aureobasidium pullulans in continuous ambulatory peritoneal dialysis

Sir,

Peritonitis is the major complication of continuous ambulatory peritoneal dialysis (CAPD). Approximately 60% of patients develop at least one episode during the first year; whereas bacterial species account for over 80% of cases, fungal peritonitis is becoming more important because of its increasing frequency and the problems with its management [1]. We report a case of peritonitis due to a rare pathogen, Aureobasidium pullulans, complicating CAPD.

A 63-year-old man with chronic renal failure secondary to tubulointerstitial disease and hypertension commenced continuous peritoneal dialysis in October 1994. Ten months later, he presented bacterial peritonitis due to S. epidermidis and S. viridans that was successfully treated with appropriate antibiotics. Ten days after, dark grey particles were observed in the transfer set and in the distal portion of the catheter (Figure 1). Examination of peritoneal fluid revealed 130 nucleated cells/mm³ with a differential of 35% PMNs and 65% mononuclear cells, but without clinical evidence of peritonitis. Microscopic evaluation of the inner walls of the catheter showed short, multisepate, thick-walled pigmented hyphae and dark conidia. Culture of the material obtained yielded fast-growing mould colonies, later identified as Aureobasidium pullulans. Antifungal therapy was started with fluconazole administered intraperitoneally (150 mg/48 h), and an oral loading dose of 2 g of fluocytosine followed by 250 mg/6 h for 4 weeks, with a favourable outcome. Four weeks after the treatment course was completed, the patient presented black dots attached to the catheter walls and turbid effluent, with a cellular count of 420 nucleated cells/mm³ (54% PMNs, 46% mononuclear cells). In cultures Aureobasidium pullulans grew again. A new course of antifungal therapy was initiated for 4 weeks with a complete resolution. The patient returned to the hospital because of his bad condition 6 weeks later, presenting true signs and symptoms of peritonitis. Since he had a peritoneal fluid cellular count of 250 cells/mm³ (30% PMNs, 70% mononuclear cells) and positive cultures, it was resolved the removal of the catheter and treatment with a new course of fluocytosine. The patient had a complete recovery, and has not had any subsequent episodes of peritonitis.

Recent reports have described a progressive decline in peritonitis rates possibly due to the introduction of technical improvements, such as plastic containers for solutions and disconnect systems; the overall incidence of infection ranges from 1 to 15% in different series [2]. In addition, the spectrum of causative organisms have changed, and fungal peritonitis has become more common. Various species of Candida are responsible for 75% cases [2]. Patients on CAPD are at increased risk of fungal infections. Renal failure and uraemia may cause, by themselves, a state of immunosuppression. There is a rupture of the cutaneous barrier, and the peritoneal catheter used for dialysis provides a port of entry for microorganisms. A history of hospitalization, recent prior episodes of peritonitis, and antibacterial therapy seems to predispose to infection [2]. Furthermore, clinically, this entity cannot be differentiated from bacterial peritonitis.

Aureobasidium pullulans is classified among dematiaceous fungi, characterized by the production of melanin pigments. They are widely distributed throughout the environment, being frequently isolated from soil, decaying vegetation, and house dust. However, there are very few reports of invasive disease caused by this microorganism. Two cases of peritoneal infection in patients on CAPD have also been previously reported [3,4].

There are no generally accepted therapeutic regimens for fungal peritonitis. Controversy centres on whether to use antifungals alone or in combination with catheter removal. The aim of an optimal treatment must be the maintenance of peritoneal catheter permeability, prevention of peritoneal adhesion formation, and sterilization of the effluent. At the present time, most authors recommend the use of antifungals, though the best regimen is not clear. In our case, we chose fluocytosine plus fluconazole because it offers several major advantages against amphoterican B, as it is the excellent bioavailability, better penetration into the peritoneal fluid, and the absence of serious toxicity. We tried to avoid removal of the catheter because there was not an important inflammatory response. We thought the origin was a contamination with the transfusion set (that had been exposed accidentally to open air), and we expected that antifungal therapy could be enough to eradicate the fungus. Only after we saw that the episode repeated and that there were clinical symptoms of peritonitis we decided to place a
new catheter. Removal of the catheter plays an important role, especially because fungal peritonitis is usually recurrent. Some authors even recommend early removal of the catheter without antifungal therapy from the beginning, unless there is persistence of clinical signs and symptoms [5]. Episodes like this are frequently overlooked, because fungal saprophytes are considered as contaminants not able to cause serious disease. We would like to draw attention to the importance of investigating the causative agent, and its antimicrobial susceptibility, because they can complicate this mode of dialysis.

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Fig. 1. Peritoneal catheter showing dark grey particles attached to the inner walls.