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Editorial Comments

The patient with IgA glomerulonephritis—what is the role of steroid treatment?

Francesco Locatelli, Lucia Del Vecchio and Claudio Pozzi

Department of Nephrology and Dialysis, Ospedale di Lecco, Lecco, Italy

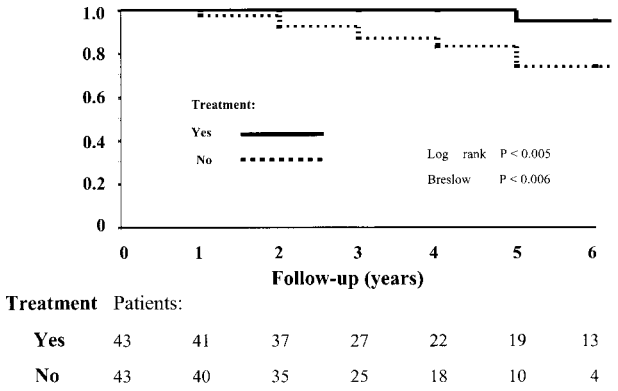
Introduction

What most disturbs us as physicians is the feeling of impotence when confronted with an important disease without established therapeutic approach. Thus it is not surprising that nephrologists were disappointed because evidenced-based treatment was not available for the commonest glomerulonephritis world-wide, i.e. IgA nephropathy (IgAN). This was particularly frustrating because, despite previous opinion to the contrary, IgAN is often progressive. Nearly 25–50% of adult patients need dialysis or renal transplantation within 10–25 years [1,2] and the outcome in children is also less than encouraging. Yoshikawa *et al.* [3] reported that 6% and 11% of 241 children with IgAN developed chronic renal failure (CRF) by 10 and 15 years respectively from the onset of the disease. It is important to keep in mind that most patients with IgAN develop end-stage renal disease (ESRD) in their middle age. This represents not only an important problem for the patient, but also a significant social and economic burden for society as a whole. The interest in finding out effective interventions is reflected by the long list of therapeutic approaches that have been suggested so far. However, these therapies have been mainly tested in a relatively small number of patients and none of them has been proved to be actually effective in the long term. The problem is further complicated by the fact that the course of IgAN is extremely variable [1,2]. Patients display very different rates of progression towards ESRD. Strangely, some patients in whom renal function is impaired at the time of diagnosis do not progress at all even after decades. This makes it very difficult to assess the effectiveness of any therapeutic approach. Only randomized trials of adequate size give sufficient statistical power to provide information.

The use of steroids in IgA glomerulonephritis

Among the long list of suggested treatments, the use of corticosteroids seems the most interesting. These potent anti-inflammatory agents have been used in the treatment of glomerular diseases for nearly 40 years and have shown variable success in patients with IgAN. In a retrospective study Kobayashi *et al.* [4] reported favourable results in patients with heavy proteinuria after steroid treatment for 18 months compared to treatment with non-steroidal anti-inflammatory drugs or anticoagulants. During a 10-year follow-up of 46 IgAN patients with normal renal function and moderate proteinuria (1–2 g/day), renal survival was significantly better in the group treated with steroids (100% *vs* 84% at 5 years and 80% *vs* 34% at 10 years) [5]. The results of randomized trials are more equivocal [6–8]. Preliminary data of a multicentre prospective trial in IgAN patients with moderate proteinuria do not support a favourable effect of steroids in a dose of 60 mg/day tapered by 10 mg every 3 months to 10 mg/day over 24 months. Only modest reduction in proteinuria and no effect on renal function were observed [6]. Short-term (12 weeks) therapy with prednisone was also found to be ineffective in 20 children [7]. More encouraging are the results of treatment with prednisone for 2 years in 13 children. Significant improvement of urinary findings (both proteinuria and haematuria) were noted in comparison with a historical group [8]. Renal biopsy performed at the end of treatment revealed a significant decrease in the activity score, without significant increase in the chronicity score [8]. Taken together, these studies suggest that a short course of steroids does not offer any particular benefit, whereas longer courses may favourably alter the evolution of IgAN. One would anticipate, however, that a longer course carries a greater risk of side effects. The above results are difficult to interpret, since they concern small studies on patients differing in terms of age (adults and children), severity of IgAN and, more importantly, degree of proteinuria. In this regard, it is important to consider that nephrotic range proteinuria is generally a maker of poor renal prognosis in IgAN. Nevertheless, a subset of patients with steroid-responsive nephrotic syndrome represent a distinct clinical syndrome (minimal change disease plus IgA deposits) which carries a low risk of progression towards ESRD, possibly because the response to the steroid treatment is good.

Correspondence and offprint requests to: Prof. Dr Francesco Locatelli, Department of Nephrology and Dialysis, Azienda Ospedale di Lecco, Via Ghislanzoni 22, I-23900 Lecco, Italy.



Treatment	Patients:						
Yes	43	41	37	27	22	19	13
No	43	40	35	25	18	10	4

Fig. 1. Renal survival in the Italian multicentre randomized trial of corticosteroids [9] estimated on the basis of reduction in GFR (estimated using the Cockcroft and Gault formula) to more than 50% below baseline values (equivalent to the primary endpoint of 100% increase of plasma creatinine levels from baseline).

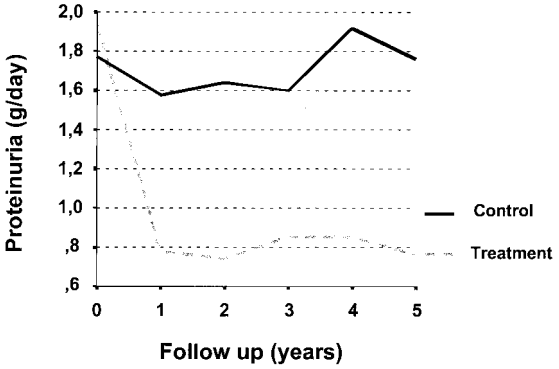


Fig. 2. Evolution of proteinuria levels in the Italian multicentre randomized trial of corticosteroids [9] in the treatment (21 patients) and control (18 patients) groups of the 5-year cohort. Mean urinary protein excretion significantly decreased in the steroid group (from 1.93 ± 0.45 g/day at baseline to 0.78 ± 0.41 g/day at 1 year), and this decrease persisted during the whole follow-up; proteinuria remained unchanged in the control group.

Italian randomized controlled trial of corticosteroids

Given that IgAN is also the commonest glomerulonephritis in Italy, it is not surprising that we were particularly interested in finding an effective and relatively safe therapy. It is well known that tubulointerstitial scarring is the most important histological factor contributing to progression in any kind of nephropathy, including IgAN. Moreover, proteinuria is the main prognostic factor in IgAN and it heavily contributes to interstitial fibrosis. Considering the importance of proliferative lesions in the acute phase of IgAN in conditioning glomerular and tubular sclerosis later on, we decided to choose steroids as first line therapeutic approach because they decrease proteinuria and possibly limit fibrosis reducing exudative lesions. According to previous clinical experiences, we performed a multicentre randomized controlled trial in order to evaluate the effects of a course of steroid (methylprednisolone 1 g i.v. for three consecutive days at the beginning of months 1, 3 and 5, plus oral prednisone 0.5 mg/kg every other day for 6 months) in comparison with supportive therapy. We studied 86 biopsy-proven IgAN patients in the early stage of the nephropathy with relatively well preserved renal function and significant proteinuria (1–3.5 g/24 h) [9]. We excluded patients with heavy proteinuria to avoid the possible confounding effect of minimal change disease with IgA deposits as mentioned above. Given the high prevalence of this form of glomerulonephritis, it is surprising that we were able to enrol only 86 patients from seven participating centres in a period of 8 years. These difficulties reflect the scepticism in many Italian nephrological centres concerning the effectiveness of steroid therapy. Against the background of disappointing results in previous small studies [6–8], the results of the Italian study are fascinating [9]. After 5 years' follow-up, renal survival was significantly better in the group with steroid treatment compared with the control group for both primary endpoints, i.e. a 50% and 100% increase from baseline plasma creatinine levels. This is seen in 17% and 21% of the patients, respectively; log-rank test $P < 0.048$ and < 0.005) (Figure 1). Three patients in the control group and none in the steroid group required dialysis. Mean urinary protein excretion also significantly decreased in the steroid group (from 1.93 ± 0.45 g/day at baseline to 0.78 ± 0.41 g/day at 1 year), and this decrease persisted throughout the follow-up, whereas proteinuria remained unchanged in the control group (Figure 2). These results are further supported by the results of the multivariate Cox analysis, using a 50% increase in baseline plasma creatinine as the endpoint. The analysis showed that steroid treatment, female gender and absence of vascular sclerosis were correlated with a lower risk of renal dysfunction. When the reduction in proteinuria between baseline and the 6-month follow-up was added to the Cox model, it was associated with better renal survival and, more interestingly, the beneficial effect of steroid treatment disappeared from the final model, thus suggesting that the effect of steroid treatment may be mainly due to reduction of proteinuria. This effect was not influenced by the administration of ACE-inhibitors, because the percentage of patients treated with these agents was the same in the two groups. Furthermore, by multivariate Cox regression analysis the use of ACE-inhibitors was not significantly correlated with a better outcome. Only the onset with macrohaematuria, which is a well-known favourable prognostic factor, was unbalanced between the two groups (more common in the control group). However, as the duration of nephropathy was not different in the two groups, the onset with macrohaematuria was not a cause of the early detection of IgAN. Even more importantly, the multivariate Cox regression analysis with the doubling of baseline plasma creatinine as the endpoint found that macrohaematuria at the onset lowered the relative risk of progression also in our trial. This could have favoured

the control group prognosis and thus possibly reduced the magnitude of the observed difference of renal survival between the two groups due to steroids. The consistency of the results is underlined by the fact that the five patients (four in the control and one in the steroid group), who violated the protocol because they were given steroids as rescue treatment after the development of persistent nephrotic syndrome, also had a significant decrease in proteinuria after 6-month steroid treatment (from proteinuria levels of, respectively, 6.8, 5.9, 10.7, 12.5 g/day to 0.3, 2.0, 2.8, 0.4 g/day).

It is worth noting that the 6-month steroid course appeared to be relatively safe, since the patients assigned to the steroid group did not experience any major side effects during the follow-up (excepting one patient who developed diabetes mellitus 2 years after treatment).

Are steroids alone enough?

Although the results of our study [9] very consistently document a positive effect of steroids on the natural course of IgAN, some problems deserve comment. First of all, by multivariate Cox regression analysis the effect of steroids was not greater than that of female gender in protecting against renal function deterioration (although the positive effect of steroids was the same in both genders). This observation suggests a possible role of genetic factors on the extreme variability of IgAN outcome that seems to be only partly modifiable by treatment. On the other hand, although the difference in renal survival was particularly striking until the third year, the risk of renal function deterioration was subsequently quite similar in both the treated and untreated patients. Furthermore, in some patients proteinuria increased again during follow-up. It is possible that the steroid effect, as well as being different in these patients, may decrease over time.

One possibility to circumvent this shortcoming would be to introduce a second course of steroids after 2–3 years. Considering that we found a very close relationship between reduction of proteinuria and preservation of renal function, urinary protein excretion might be a useful indicator for the necessity to start another course of therapy. However, the results of our trial [9] suggest that the first 6-month course of therapy is probably not sufficient to ensure stable remission and completely quench the immune and inflammatory response. As a consequence, the development of a certain degree of sclerosis probably occurs and this may reduce the effectiveness of a second course of therapy, at least in the long term.

Another possibility is to provide more aggressive treatment in the early phase of the nephropathy, in order to reverse proliferative lesions as much as possible and prevent the development of fibrosis.

Strangely enough, an uncontrolled retrospective study of combined treatment with corticosteroids for 18 months and azathioprine for 24 months showed that the treatment was only effective in patients with impaired renal function [10]. However, the lack of randomization led to a severe bias concerning the choice of the patients to be treated (higher risk patients). Combined treatment with prednisolone for 2 years and cyclophosphamide for 3 months, followed by azathioprine was also shown to be effective in preserving renal function at 3 years in 37 randomized adult IgAN patients with moderate rate of CRF progression [11]. In children with newly diagnosed IgAN, Yoshikawa and Ito [12] recently reported that treatment with prednisolone, azathioprine, heparin–warfarin, and dipyridamole for 2 years was effective in reducing proteinuria (from 1.35 ± 1.01 to 0.22 ± 0.31 g/24 h), serum IgA levels and the intensity of mesangial IgA deposits ($P = 0.02$), with no change in the percentage of sclerotic glomeruli. The relatively short follow-up period (2 years) after an 'early' diagnosis did not allow to establish the effect of treatment on renal function deterioration (only one patient in the heparin–warfarin and dipyridamole group developed CRF). Although relatively few serious side effects were reported, in our opinion the 2-year treatment with azathioprine in children affected by a low-progressive disease rises great concern about long-term adverse effects, such as the risk of gonadal toxicity and oncogenicity (although the experience on transplanted children is reassuring). Considering that the combination of heparin–warfarin and dipyridamole was completely ineffective, it is surprising that the authors planned a new controlled trial aimed at comparing the effects of prednisolone, azathioprine, heparin–warfarin, and dipyridamole with prednisolone alone.

In conclusion, in our opinion the key question is not so much whether prednisolone and azathioprine are essential components of the combined therapy, but whether prednisolone alone is enough to ensure stable remission and if azathioprine can add further benefit. Only if azathioprine (possibly at a lower dose) has been proven to provide substantial long-term benefit would we no longer object to use this agent in children. This is exactly the reason why we planned a new long-term randomized controlled adequately-sized trial to evaluate the role of low-dose of azathioprine added to steroids in adult patients with IgAN.

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Editor's note

Please see also the Personal Opinion paper by Yoshikawa (pp. 1097–1099 in this issue)

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Thin basement membrane—do we have a window for understanding the molecular pathogenesis?

Kai-Olaf Netzer, Stefan Seibold and Manfred Weber

Medizinische Klinik I, Krankenhaus Köln-Merheim, Köln, Germany

Introduction

Thin glomerular basement membrane (GBM) is a frequent biopsy finding, occurring in 5–10% of renal biopsy series. Biopsies of kidney allografts indicate that the frequency of thin GBM may even reach up to 9% in the general population [1], making it the most common renal condition.

Symptoms

Clinically, individuals with thin GBM almost always present with microscopic haematuria. The family history is positive for haematuria in more than half of the cases, while systematic urinalysis reveals haematuria to be present in more than 90% of the families, affecting half of all relatives. Thin GBM therefore seems to be an autosomal dominant condition with regard to haematuria. Other symptoms include macroscopic haematuria, loin pain and low-degree proteinuria. The clinical course of thin GBM is usually benign with an excellent prognosis, which has led to the clinical nomenclature 'benign familial haematuria'.

Thin GBM and Alport syndrome

There have been reports of premature glomerular obsolescence and an increased rate of renal function deterioration with age in patients with thin GBM disease [2], possibly indicating an impaired stability of the thin GBM. In some families, the occurrence of extra-renal symptoms such as deafness (10%) has been reported. Electron-microscopy occasionally reveals not only extreme thinning of the GBM, but also some lamellation. These clinical and pathological features resemble those of early Alport syndrome, in which the underlying structural defect of basement membranes is caused by mutations in type IV collagen genes. Given the similarities to early Alport syndrome, it has long been suspected that thin GBM might be related to mutations in the autosomal type IV collagen genes.

Type IV collagen is a major structural component of all basement membranes, providing a molecular scaffold in the form of a supramolecular network. Six genetically distinct type IV collagen α-chains (α1–6(IV)) have been identified, four of which, the so-called novel chains (α3–α6(IV)) have been implicated in the pathogenesis of Alport syndrome. Homozygous mutations in the *COL4* genes on chromosome 2 encoding the α3 and the α4 chains cause the autosomal form of the disease. While homozygotes present with the 'classical' symptoms of Alport syndrome, heterozygote carriers present with microscopic haematuria in $>60\%$ of the cases, proteinuria in $>10\%$, and hearing loss in $>20\%$. The incidence of end-stage renal failure is very low. Thus, symptoms of autosomal recessive Alport carriers and patients with benign familial haematuria are practically identical.

Evidence from family studies

Lemmink *et al.* [3] studied a family with benign familial haematuria in which they found linkage to the

Correspondence and offprint requests to: K.-O. Netzer, Medizinische Klinik I, Krankenhaus Köln-Merheim, Ostmerheimer Str. 200, D-51109 Köln, Germany.

COL4A3/A4 gene locus. They also identified a *COL4A4* mutation which caused the phenotype of benign familial haematuria in heterozygous carriers, thereby establishing for the first time that type IV collagen mutations may indeed represent the molecular basis for thin GBM disorder as well as Alport syndrome.

The hypothesis that benign familial haematuria represents a carrier status of autosomal Alport syndrome, at least in some cases, is supported by the molecular characterization of other families [4,5]. In one consanguineous family, the elimination of a *COL4A4* exon from the mRNA was associated with haematuria in heterozygotes, while homozygotes presented with hearing loss and more advanced impairment of renal function, fulfilling the diagnostic criteria of Alport syndrome [4]. Preliminary results of a recent linkage study of 13 families with biopsy-proven thin GBM showed that ~50% of the cases are linked to the *COL4A4/A3* gene locus [6], while linkage to *COL4* loci could be excluded only in approximately one-third of the cases.

Conclusions

Taken together, these findings indicate that thin GBM is a pathogenetically heterogenous condition, a substantial portion of which is related to the autosomal type IV collagen gene locus on chromosome 2. The identification of heterozygous *COL4A4* mutations in single families with benign familial haematuria phenotype confirms that benign familial haematuria may represent a carrier status of autosomal recessive Alport syndrome.

It is conceivable that *COL4A4* mutations affecting only one allele will not abolish the expression of the respective type IV collagen chain. This is in line with results from immunohistochemical and immunogold studies indicating that the thin GBM contains all the normal type IV collagen chains (α1–5(IV)) [7]. Possible effects of *COL4A4* mutations in benign familial haematuria could be a decreased content of type IV collagen novel chains in the GBM, a reduced degree of cross-linking in the type IV collagen network, and ultimately decreased GBM thickness and stability.

While the molecular pathogenesis of thin GBM needs more study, we believe that the window for its understanding has now been opened, paving the way for improved diagnostic testing in the future. It is hoped that this will eventually facilitate the approach to patients with isolated haematuria for clinicians.

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Night time blood pressure in diabetic patients—the submerged portion of the iceberg?

Guntram Schernthaner, Eberhard Ritz[1], Thomas Philipp[2] and Reinhard G. Bretzel[3]

First Medical Department, Rudolfstiftung, Vienna, Austria, [1]Department of Internal Medicine, Ruperto Carola University, Heidelberg, [2]Department of Internal Medicine, Division of Nephrology and Hypertension, Essen and [3]Department of Internal Medicine III, Justus Liebig University Giessen, Germany

Introduction

Nowadays there is no longer any doubt that elevated blood pressure is of overriding importance for the development of renal and cardiovascular complications in diabetes mellitus. This is true even for values of blood pressure which are in the upper range of normotension according to the WHO definition. What is less well known is the fact that it is not only the average level of blood pressure, but particularly an abnormal circadian profile which determines diabetic complica-

Correspondence and offprint requests to: E. Ritz, Med. Universitäts Klinik, Bergheimer Str. 58, D-69115 Heidelberg, Germany.

tions. This cannot be assessed properly using clinic measurements or even self-measurements of blood pressure by the patient. Its evaluation necessitates ambulatory blood pressure measurement. This group of Austrian German diabetologists and nephrologists feel that ambulatory blood pressure measurement is not utilized sufficiently. We marshal some evidence which illustrates the relevance of ambulatory blood pressure measurement in diabetic patients, particularly in diabetic patients with renal disease.

The value of blood pressure reduction in diabetic patients

Arterial hypertension is a decisive risk factor for the initiation and/or progression of diabetic microangiopathy and macroangiopathy. Hypertension is 2–3 times more frequent amongst diabetic compared with non-diabetic individuals. The prevalence of hypertension increases with increasing rates of urinary albumin excretion [1]. The overriding importance of lowering the blood pressure for the reduction in the rate of cardiovascular complications in diabetic patients has been documented by several recent studies. In 1996, the SHEP study (Systolic Hypertension in the Elderly Program) documented that in elderly patients with type 2 diabetes and isolated systolic hypertension, low doses of diuretic treatment compared with placebo reduced the rate of cardiovascular events by 34% [2]. The absolute reduction of risk by active treatment compared with placebo was twice as high in diabetic patients compared with non-diabetic patients, i.e. 101/1000 *vs* 51/1000 randomized patients during a 5-year follow-up. This observation illustrates that the absolute risk is markedly higher in diabetic patients. The excess risk of hypertensive diabetic patients recently was also confirmed in the HOT (Hypertension Optimal Treatment) study [3]. When a target blood pressure of 80 mmHg was aimed at instead of 90 mmHg, the rate of cardiovascular events and fatalies was halved. This was not demonstrable in the non-diabetic population. A most impressive reduction of incidences of death recently could also be demonstrated in the UKPDS (UK Prospective Diabetes Study) [4,5]. In patients with intensified compared with regular blood pressure lowering, i.e. BP 144/82 mmHg compared with 154/87 mmHg, the risk was markedly lower both for macrovascular and microvascular complications including renal events. No significant difference could be demonstrated between atenolol and captopril.

Importance of ambulatory blood pressure measurement

Recent studies clearly indicate that in diabetic patients, measurement of ambulatory 24 h blood pressure is a much better predictor of microvascular and macrovascular complications than conventional blood pressure measurement. Such superiority is presumably due to better reproducibility, complete assessment of the circadian profile and exclusion of the 'white coat phenomenon' [6]. Sturrock [7] measured 24 h blood pressure in diabetic out-patients and showed that the values of clinic blood pressure were wrong for systolic blood pressure in 82% and for diastolic blood pressure in 55% of the patients. Nielsen *et al*. [8] noted that 'white coat' hypertension was present only in 8 and 9% of the microalbuminuric and macroalbuminuric diabetic patients studied, whereas this phenomenon was present in 23% of normoalbuminuric patients. Equiluz-Bruck *et al*. [9] examined 72 patients with type 2 diabetes and found that an absent nocturnal decrease in blood pressure (non-dipping) was more frequent in diabetic compared with non-diabetic hypertensive individuals. In patients with type 2 diabetes, non-dipping, i.e. a nocturnal decline of BP $< 10\%$, was significantly correlated with the albumin excretion rate. Non-dipping was found in 80% of patients with macroalbuminuria and 74% of patients with microalbuminuria, but only in 43% of patients with normoalbuminuria. The authors argued that non-dipping might contribute to the high cardiovascular mortality of diabetic patients with microalbuminuria or macroalbuminuria.

Recently, Poulsen *et al.* [10] documented that 24 h blood pressure is correlated with albumin excretion rate even in type 1 diabetic patients without diabetic renal disease, i.e. in normoalbuminuric patients. An attenuated decline in nocturnal blood pressure is apparently an early indication of end-organ damage: Poulsen *et al*. found that in such normoalbuminuric patients with type 1 diabetes, higher night time blood pressure values and failure of blood pressure to decrease during the night were correlated with early appearance of retinopathy [10]. We emphasize that in these patients diabetic nephropathy was excluded because of the normal urinary albumin excretion rate. Non-dipping is also a predictor for the development of microalbuminuria: in a 3-year follow-up study, Poulsen *et al*. [11] documented that patients with type 1 diabetes who were non-dippers had a significantly higher risk of developing microalbuminuria than did patients with normal dipping of blood pressure during the night. Based on these results, one must conclude that an absent nocturnal decrease in blood pressure is a first important indicator of evolving renal damage. Recognition of the risk will allow timely therapeutic intervention [12].

Of particular interest is the study of Nakano *et al*. [13] who found a relationship between the circadian blood pressure profile and the occurrence of fatal and non-fatal vascular events in patients with type 2 diabetes. The authors followed 325 patients over 3–4.5 years. If these diabetic patients had a 'reversed' circadian blood pressure profile, the risk of dying was 20-fold higher than patients who had a normal decrease in blood pressure during the night ($P = 0.0001$). Of particular note is the observation that most sudden deaths or strokes occurred during night time or early morning. This observation is in line with the results of the ISIS-2 study [14] which documented that the rate

of myocardial infarctions increases dramatically between 6 and 8 a.m. For a long time, it had been suspected that genetic predisposition to essential hypertension determines the risk of patients with type 1 diabetes to develop diabetic nephropathy. Using ambulatory blood pressure measurement, Fagerudd *et al.* [15] could clearly show that parents of patients with type 1 diabetes and nephropathy had a significantly higher prevalence of hypertension than parents of propositi with type 1 diabetes and without nephropathy. These studies illustrate the high sensitivity of ambulatory blood pressure measurements for evaluating the renal risk. Similar observations have also been made in type 2 diabetes [16].

Mechanisms of non-dipping

The mechanisms underlying non-dipping of blood pressure have not been elucidated. Initially, it had been assumed that non-dipping was an indication of established end-organ damage. Recent studies showed that non-dipping is demonstrable very early on before end-organ damage has occurred. Chen *et al.* [17] examined glucose tolerance, insulin secretion and hormones relevant for blood pressure regulation in 15 non-diabetic normal weight patients with essential hypertension. These parameters were correlated with the 24 h blood pressure profile. In the oral glucose tolerance test, non-dippers had significantly higher blood glucose concentrations and significantly lower insulin concentrations compared with dippers. Non-dippers had a higher heart rate during night time. Norepinephrine and dopamine concentrations were also significantly higher than in non-dippers. These findings suggest that even in early stages of type 2 diabetes, an association exists between insulin resistance, beta cell dysfunction and non-dipping. The hypothesis has been advanced that a high sympathetic tone during night time plays an important role.

Night time blood pressure—a predictor of cardiovascular and renal events?

Because of the close relationship of hypertension and diabetic nephropathy, and because of their high risk of cardiovascular events, the measurement of ambulatory blood pressure is of particular importance in diabetic patients, as single conventional blood pressure measurements are highly variable, yielding variations in systolic and diastolic blood pressures of 50 or 30 mmHg, respectively. In contrast, under standardized conditions, the coefficient of variation is very low for 24 h blood pressure measurements, i.e. 2–3% for the 24 h blood pressure measurement and 5–6% for the night time/day time ratio.

Currently, several studies are underway to analyse whether lowering nocturnal blood pressure reduces the frequency of end-organ damage and improves the prognosis in diabetic patients.

Conclusions

There is no doubt that 24 h blood pressure measurement in diabetic patients with renal disease yields information which goes far beyond what can be obtained with clinic blood pressure measurement or self-measurements. Particularly night time blood pressure values permit a much more focused antihypertensive treatment in diabetic patients with nephropathy as discussed elsewhere [18]. It is the opinion of the authors that ambulatory blood pressure measurement is indispensable for optimizing antihypertensive treatment in diabetic patients with nephropathy.

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Does hydration prevent radiocontrast-induced acute renal failure?

Christiane M. Erley

Department of Internal Medicine, Section for Nephrology and Hypertension, University of Tuebingen, Tuebingen, Germany

Introduction

A decline of renal function after the administration of contrast media (CM) is a frequent cause of hospital-acquired acute renal failure. This so called contrast-media-induced nephropathy (CMIN) includes a haemodynamic response to contrast media and tubulotoxicity. Although the clinical features of CMIN have been well described, uncertainties concerning the prophylaxis and clinical relevance of this form of nephrotoxicity persist. The purpose of this article is to review the role of a hydration strategy in the prevention of this condition.

From a theoretical point of view prehydration of patients may have the following beneficial effects on the kidney:

- decreased activity of the renin–angiotensin system,
- downregulation of the tubuloglomerular feedback,
- augmentation of diuresis and sodium excretion,
- dilution of the contrast media and thus prevention of renal cortical vasoconstriction,
- reduced pre-constriction of the vessels,
- avoidance of tubular obstruction, and
- reduction of endothelin and other intrarenal vasoconstrictive mediatiors (e.g. vasopressin).

Historical background

Approximately 30 years ago several studies documented that dehydration accentuates the risk of renal failure especially in patients with diabetes mellitus or pre-existing renal failure [1]. The incidence was higher in summer at a time when no special hydration was performed and patients had to thirst before excretory urograms in order to maximize the concentration of contrast media in the urinary tract. Sometimes patients were given laxatives before intravenous pyelography, a factor further aggravating dehydration. Observations comparing hydrated patients with a historical population gave the first clues that a fluid load might prevent CMIN [2–4]. So far, no controlled systematic study has been published addressing the question which sort of fluid, how long, how often and how much should be given in order to minimize the risk of CMIN.

Experimental studies

In a rabbit model of CMIN involving low-sodium diet and administration of indomethacin, the infusion of isotonic saline or isotonic mannitol (both given at a rate of 20 ml/h/kg, equal to 4% of the animal's body weight over a 2-h period) parallel to the infusion of the contrast media was not able to prevent acute renal failure, while pre-treatment of the animal with chronic high sodium intake and DOCA administration did [5]. As plasma renin activity is reduced by administration of DOCA as well as by an acute infusion of saline and mannitol, the authors concluded that apart from lowering of intrarenal renin and plasma renin activity, the increase of urinary sodium and solute excretion *per se* (and probably the plasma volume expansion) contributed to the prevention of CMIN. These data were confirmed by our own study in rats with high intravascular resistance due to chronic NO inhibition. DOCA pre-treatment completely reversed the haemodynamic response to contrast media [6]. Yoshioka *et al.* [7] showed that water-deprived rats (72 h) had reduced activities of catalase and superoxide dismutase and were highly sensitive to the application of diatrizoate which caused a significant and persistent fall in GFR 72 h after CM application. After injection of saline water-deprived rats gradually normalized GFR by 72 h.

Clinical studies

Most studies dealing with the issue of hydration in the prevention of CMIN addressed the role of mannitol

Correspondence and offprint requests to: Christiane M. Erley MD, University of Tuebingen, Medical Section III, Section of Nephrology and Hypertension, Otfried-Mueller-Str. 10, D-72076 Tuebingen, Germany.

or the role of vasodilators such as dopamine, atrial natriuretic peptide, Ca antagonists, or ACE inhibitors with regard to the protection of the kidney from contrast media damage [8–13]. The authors found that hydration alone was as effective or even better than additional administration of hypertonic mannitol or the administration of one of the vasodilative agents. Other investigators compared results in patients submitted to special hydration protocols with historical control groups [2,3] or data reported in the literature [4,14,15] whereby with hydration alone the incidence of acute renal failure was lower. So far only one controlled, randomized study compared saline administration alone (0.45% saline over 24 h, starting 12 h before administration of radiocontrasts) with mannitol (25 g of mannitol given 60 min before administration of radiocontrasts) or frusemide (80 mg i.v.) [8]. In this study administration of saline alone was the most successful strategy. In numerous studies dealing with the nephroprotective effect of non-ionic contrast media prehydration of the patients was included in the protocol [16,17], but patients with cardiac failure, liver cirrhosis or oedema have mostly been excluded from the studies in order to avoid overhydration.

Which fluid and when to start?

Most investigators administer 0.45% saline in combination with 5% dextrose intravenously in various amounts (around 1000–1500 ml starting 12 h before administration of radiocontrasts). There is no controlled study which assessed oral hydration in these patients. How long hydration should be continued has also not been investigated so far. In accordance with the experimental data good results in humans have been obtained with hydration prior to and up to 12 h after contrast media exposure [2,4,8]. Only a minor beneficial effect could be seen when fluid was administered during the procedure [3,15]. From a theoretical point of view the use of hyperosmolar fluids (such as 15% mannitol) in addition to the administration of the hyperosmolar contrast media may have adverse effects. Therefore it is not surprising that most studies failed to observe a beneficial effect of mannitol in this setting [2,8,9].

Use of diuretics?

No conclusive evidence is available to support a protective role of loop-active diuretics in regard to the prevention of CMIN. From the theoretical point of view it has been claimed that reducing the 'workload' of the tubular cells by decreasing the rate of sodium reabsorption might be tubuloprotective. Additionally there might by a dilution effect by an increment of diuresis after frusemide. Most investigators dealing with this point showed no benefit or sometimes even worse results after administration of frusemide [8,18,19]. The adverse effect of frusemide could be due to reduction of cortical resistance causing redistribution of renal blood flow and reduced perfusion of the medulla. In combination with the contrast-media-induced vasoconstriction, partial pressure of oxygen in the medalla could thus be reduced below a critical point. Consequently, if it is used at all, frusemide should be administered with caution, rigorously avoiding dehydration, which, by itself, would definitely enhance the nephrotoxicity of contrast agents.

Conclusion

So far no controlled prospective study addressed the issue, which hydration strategy is optimal in order to prevent CMIN. Presently, it seems appropriate to start hydration at least 12 h before administration of contrast media in order to induce volume expansion with concomitant suppression of the renin system. This could be continued for 12–20 h after the procedure. The best route of fluid administration, the amount and the type of fluid have to be clarified. Whether this strategy is safe in patients with heart failure, liver cirrhosis and edema has to be shown in future studies. The use of loop-active diuretics should be avoided as no benefit in preventing CMIN has been proved and hypovolaemia could be enhanced.

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Vitamin D receptor polymorphisms as a determinant of bone mass and PTH secretion: from facts to controversies

Jordi Bover[1] and Ricardo J. Bosch[2]

[1]Nephrology Department, Hospital Prínceps d'Espanya, CSUB, L'Hospitalet de Llobregat, Barcelona, Catalonia and [2]Physiology Department, University of Alcalá, Alcalá de Henares, Spain

Introduction

During recent years, powerful molecular biology techniques such as restriction enzymes and positional cloning have been used to identify genetic diseases even before the responsible genes were characterized. However, clinical studies have shown that bone mineral density (BMD) is under genetic control, probably polygenic in origin, and several candidate genes, namely oestrogen and vitamin D receptors (VDR), as well as collagen type I α 1 among others, may mediate important differences in bone mass and bone metabolism. Since the first description by Morrison *et al.* [1], several groups have shown that genetic polymorphisms at the 3′-untranslated region of the VDR gene may account for at least some of the genetic variation in bone mass. These polymorphisms are defined by the presence or absence of a restriction site for the enzymes *Bsm*I, *Apa*I and *Taq*I. Since that initial report, VDR gene polymorphisms have been associated with BMD, peak bone density, bone turnover and the serum levels of some biochemical bone markers, as well as the rate of bone loss, risk of osteoporotic fracture and the relative response to several treatments of osteoporosis such as vitamin D or calcium.

VDR polymorphisms in patients without renal failure

According to the most widely analysed *Bsm*I restriction site—*B* absence, *b* presence of a cleavage site—several studies have documented that in patients without chronic renal failure (CRF), the presence of two copies of the allele *b* (genotype *bb*) is associated with a greater BMD than the heterozygous genotype *Bb*, whereas the genotype *BB* is associated with the lowest BMD [1]. Generally speaking, the *BBAAtt* genotype has usually been related to a lower bone mass. However, there is not general agreement and several reports have failed to confirm such a relationship. A recent meta-analysis provided evidence for an effect of the VDR polymorphisms on BMD, but it was quantitatively modest [2]. It has also been shown that environmental factors may influence the effect of genetically determined BMD. Thus, VDR genetic polymorphisms have been linked to differences in intestinal fractional calcium absorption. As such, individuals with the *bbaaTT* haplotype showed a higher rate of radiocalcium absorption [3]. Conversely, individuals with the *BB* genotype had a lower efficiency of calcium absorption after dietary calcium restriction and had a lower BMD than those with the *bb* genotype [4]. This finding would be consistent with the presence of functional differences in the intestinal VDR among different VDR genotypes. However, the mean increase of BMD after treatment with vitamin D was significantly higher in individuals with the *BB* and *Bb* genotypes compared with the *bb* genotype [5]. A more pronounced suppression of PTH concentration by calcitriol has also been described in individuals with the *bb* genotype. Furthermore, VDR polymorphisms have been associated with urinary calcium excretion, but in this specific study they were not related to BMD [6]. Consequently, VDR polymorphisms seem to represent one of the genetic factors affecting BMD, but they account only *partially* for the overall genetic effect on bone mass and this effect is not observed in all the screened populations.

VDR polymorphisms in primary and secondary hyperparathyroidism

The VDR genetic polymorphisms have also been linked to the development of primary and secondary hyperpa-

Correspondence and offprint requests to: Jordi Bover MD, Nephrology Department, Hospital Prínceps d'Espanya, CSUB, C/Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain.

rathyroid disorders. Whereas the *BBAAtt* genotype has usually been related to lower BMD in non-renal populations, an increased prevalence of the polymorphic VDR alleles *b*, *a* and *T* has been demonstrated in sporadic *primary* hyperparathyroidism (HPT). The VDR haplotype *baT* seems to be a risk factor for parathyroid adenomas, possibly by interfering with the inhibitory action of calcitriol [7,8]. Thus, in patients who were homozygotous the *baT* alleles parathyroid tumours exhibited lower VDR mRNA and higher parathyroid hormone (PTH) mRNA levels than those harbouring the *BB*, *AA* or *tt* genotypes [7]. In contrast, the *BAt* haplotype has recently been shown to be under-represented in primary HPT but is related to larger parathyroid lesions, as well as a less deranged calcium sensor protein expression and parathyroid cell function. In these patients, primary HPT may be associated with genetic determinants, which may act mainly by altering the regulation of cell proliferation, rather than the calcium-sensing mechanism of the parathyroid cells [8].

However, data correlating VDR polymorphisms with *secondary* HPT and renal bone disease are sparse. Higher PTH levels in individuals with the *bb* genotype and lower PTH levels in the *BB* genotype have been reported in patients undergoing dialysis [9,10]. In a large haemodialysis population, Tsukamoto *et al.* [9] found that the *bb* genotype correlated with higher PTH levels than did the *BB* genotype. This finding has been confirmed by others. In addition, Fernandez *et al.* [10] have independently described the presence of a higher frequency of the *BB* genotype and the *B* allele in their low PTH group. Both PTH and osteocalcin levels have also been reported to be higher in the *aa* and *bb* genotype, and preliminary data showed that the *aa* genotype may be linked to an acute higher PTH increase when serum calcium was lowered during dialysis. However, many other groups have been unable to relate the VDR genotype with the severity of secondary HPT, VDR mRNA levels or the pattern of renal osteodystrophy. All these inconsistencies, and the poor reproducibility of results among different populations (either with or without CRF), may be caused by sampling bias, ethnicity (the prevalence of the suspected high-risk genotypes is very low in some populations and this factor would limit the statistical power of analysis), confounding environmental and dietary influences, age, obesity, physical activity, sex, menopausal status or other yet unidentified factors.

VDR polymorphisms in renal transplantation

The genetic expression of VDR alleles has also been studied in renal transplant patients to analyse whether these alleles may predict post-transplant loss of bone mass [11]. In this context, the *bb* genotype was linked to a better rate of bone recovery between 3 and 12 months after grafting, independent of the prevailing PTH levels [11]. Thus, patients with the *bb* genotype are, to some extent, protected against the common bone loss occurring after renal transplantation, since those exhibiting the *B* allele had lower BMD from the third month after grafting. These results are in agreement with those initially presented by Morrison *et al.* [1] in osteoporotic populations, as well as some preliminary data described in orthotopic hepatic transplantation (Guardiola *et al.*, unpublished data). Therefore, it seems likely that the effect of the VDR genotype on BMD may become more evident under challenging conditions (such as calcium restriction or following corticosteroid treatment). Nevertheless, in CRF patients, there are so many interrelated confounding variables, affecting both bone and parathyroid gland function, that the relative effect of a specific genetic background may be easily masked by other environmental or physiopathological factors with a stronger direct influence on those tissues. As a result, it seems clear that VDR polymorphisms are not one of the main determinants of BMD in patients undergoing dialysis, although it may affect bone mass in some subgroups of patients or in certain populations.

Physiological consequences of VDR polymorphisms

It is worth mentioning that the previously stated restriction enzymes act in an untranslated region of the DNA, and so none of the restrictive polymorphisms change the translated protein. Consequently, it is difficult to establish a link between the presence of the different alleles and differences in VDR expression or functionality. It was previously thought that the *b* allele was linked to a decreased transcriptional activity or VDR mRNA stability, and that such reduction of VDR expression in the parathyroids of '*bb*' patients could lead to decreased vitamin D action (calcitriol resistance) and contribute to parathyroid cell proliferation. On the contrary, it was possible that the *B* allele could be associated with an increased VDR mRNA expression or stability. Although the *baT* alleles have been shown to be linked to lower VDR and higher PTH mRNA levels in *primary* HPT [7], VDR polymorphisms do not seem to affect the abundance of the VDR mRNA in other studies and recent data do not confirm allele-specific differences in mRNA [12,13]. As a consequence, the mechanistic association between VDR polymorphisms and their phenotypic consequences is not yet clear. A recently described polymorphism at the first of the two potential translation initiation sites (ATG) in the promoter region of the VDR gene (defined as starting codon polymorphism by the *Fok*I restriction enzyme) may provide more helpful information [14]. The T/C polymorphism defines two distinct VDR protein lengths with apparently distinct affinity for its ligand and therefore different biological activity. However, only preliminary and inconsistent information is currently available on *Fok*I polymorphisms in patients with CRF. Moreover, inheritance of bone mass is probably under polygenic control and a linkage disequilibrium effect between the

VDR gene and any other disease-causing gene loci nearby seems likely. The fact that to date no differences in the quantity, properties or cellular responsiveness to calcitriol have been found, which significantly correlate with VDR genotypes argues in favour of such a hypothesis.

Conclusion

In summary, the relevance of VDR polymorphisms are still a matter of debate since correlations are poorly reproducible. Classic VDR polymorphisms seem to have a modest impact on BMD, but their role in determining calcitriol resistance and PTH levels in patients with CRF is inconsistent. In any case, in this context known VDR polymorphisms do not seem to be the main determinants of BMD, although they might have an effect in some subpopulations. A better characterization of encoding DNA polymorphisms and the regulatory regions of the gene, as well as their intrinsic relationship with new polymorphisms, may help to resolve these controversies. Currently no clear-cut genetic parameter is available that could allow us to manage patients with osteopenia or renal osteodystrophy.

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How to identify the haemodialysis access at risk of thrombosis? Are flow measurements the answer?

Peter J. Blankestijn and Johannes H. M. Smits

Department of Nephrology, University Hospital Utrecht, The Netherlands

Introduction

It is clear to most clinicians and even recognized by the NKF-DOQI task force that the primary choice of vascular access is an arteriovenous fistula (AVF), typically connecting the radial artery with the cephalic vein system [1]. However, many patients depend on an implanted graft for their access to the blood stream. Usually the graft is manufactured from polytetrafluoroethylene (PTFE). In some populations in the United States PTFE dialysis grafts account for as many as 83% of access placements [1]. The European Dialysis and Transplantation Association (EDTA) does not collect data on this issue. A survey on 1 January 1997, which included the majority of the approximately 3000 haemodialysis patients of The Netherlands found that 30% of this population was dialysed using a graft, mostly PTFE grafts.

Correspondence and offprint requests to: Peter J. Blankestijn, Department of Nephrology, Room F03.226, University Hospital, PO Box 85500, 3508 GA Utrecht, The Netherlands.

The leading cause for graft failure is thrombosis, responsible for considerable morbidity and even mortality. This Comment focuses on strategies applicable in clinical practice which may help to reduce thrombosis rate of PTFE grafts.

Identifying grafts at risk of thrombosis

Fully matured native AVFs seldom clot. In contrast, graft thrombosis occurs at a rate of 0.5–1.3 events per patient year [1–4]. A substantial number of studies have indicated that thrombosis is associated with the presence of one or more stenotic areas, which are, in the majority of cases, located at the venous anastomosis or elsewhere in the venous outflow tract [5–9]. It is now clear that timely treatment of these stenoses before thrombosis occurs, reduces the thrombosis rate.

Physical examination

A number of findings may indicate the presence of venous stenosis. These include: oedema of the extremity where the vascular access is located, prolonged bleeding after venipuncture, and changes in pulse or thrill in the graft [10]. Only few studies have systematically assessed the value of physical examination. Especially auscultation and palpation of the graft have turned out to be helpful in localizing stenotic lesions in some studies [7–10]. Although physical examination is simple and inexpensive, many clinicians will feel that its value is limited because results are highly investigator dependent.

Imaging techniques

Because of the strong association between thrombosis and the presence of stenosis, some have advocated screening patients for stenoses, in order to be able to treat these anatomical abnormalities before they cause thrombosis. Angiography and magnetic resonance imaging provide information concerning the anatomy but are unsuitable for routine screening. Doppler ultrasonography has been used and found reasonably effective for localizing stenoses. The concept that correction of asymptomatic stenoses would improve patency, was recently tested. Lumsden *et al.* randomized patients with $>50\%$ stenosis to have either a percutaneous transluminal angioplasty (PTA) or no PTA [11] and found that outcome did not differ in the two groups. These negative results are important. The authors clearly showed that selection of patients for angioplasty exclusively on the basis of anatomical criteria does not result in a reduction of thrombosis rate.

Venous pressures

Information on the functional status of the graft can be obtained from flow and pressure. Obviously, grafts do not autoregulate. Therefore, the flow is determined by the blood pressure difference and the resistance over the vascular access tract. Resistance is determined by the anatomy of the supplying artery, the graft and the draining venous system. Both flow and pressure can be used as an index of resistance.

Venous pressure measured by the dialyser increases when resistance increases due to the presence of a stenosis. It is important to realize that only the resistance of the flow tract downstream from the venous needle will be reflected by venous pressure. Most stenoses are located at the venous anastomosis or elsewhere in the venous outflow tract [5–9]. Indeed, Schwab *et al.* [12] and Besarab *et al.* [13] showed in their landmark papers that venous pressure is an easily applicable method to select patients at risk of thrombosis. They showed that treating stenoses in patients identified in this way indeed resulted in a reduction of thrombosis rate to approximately 0.20 per patient year. This very low number needs to be looked at with some caution, because the data included native AVFs which have a spontaneous thrombosis rate that is much lower than in AV grafts. Cayco *et al.* used venous pressures for surveillance of grafts [3]. They reported a thrombosis rate of 0.29 events per year. Thus, it seems safe to conclude that venous pressures are helpful in the effort to reduce thrombosis rate.

Access flow

High resistance will lead to low flow. Older studies showed that low graft blood flow is associated with an increased risk of thrombosis (summarized in [14]). However, the earlier techniques used to measure flow are unsuitable for routine use in clinical practice. Recent technological developments have allowed the introduction of an interesting and potentially valuable new tool. Krivitski [15] showed that flow can be measured relatively easily in grafts by an ultrasound dilution technique (Transonic HD01 Hemodialysis Monitor; Transonic Systems, Inc., Ithaca, NY). We and others have provided evidence that this new technique indeed enables us to quantify graft flow with sufficient accuracy [14,16,17]. Graft flow ranges from <100 ml/min to >2000 ml/min. We found in 166 grafts four cases with flows >2000 ml/min [14,18,19], whereas May *et al.* [8] reported a flow >2000 ml/min in nine of 87 PTFE grafts.

In a subsequent study we confirmed that patients with stenoses in the venous outflow tract show on average a higher venous pressure and lower flow than those without stenosis [18]. However, venous pressure did not correlate with graft flow. In other words not all patients with high venous pressure had low flow, indicating that not all patients who are at risk of thrombosis can be identified by venous pressure measurements. We also showed that inflow resistance (that is resistance of the flow tract upstream of the venous needle) comprises a substantial and very variable part of total graft resistance. This inflow resistance is not reflected by venous pressure measurements.

In our next study we investigated the hypothesis that in clinical practice flow measurements indeed give additional information to venous pressures [19]. In a group of patients who were controlled and selected for further diagnostic and therapeutic interventions by venous pressures, thrombosis still occurred and did so in patients who had a flow <600 ml/min.

Thus, we have the theoretical basis for the assumption that flow measurements are better than the only validated method for access surveillance, i.e. venous pressure measurements. We have to realize that surveillance using venous pressures is without extra cost and results are very easy to obtain. Therefore, the question is whether flow measurements really confer additional benefit in patients who are monitored using venous pressure measurements. In other words, when simple clinical variables such as venous pressures are used, is there any additional benefit when periodic flow measurements are added to the surveillance protocol? Such data are not currently available.

Introduction of periodic flow measurements means more work for the dialysis staff and the need for a separate device. It is likely that more interventions, mainly angiographies and PTAs, will be done when flow measurements are added to venous pressure measurements. This increases cost. PTA means substantial vascular injury and it is possible that frequent PTA of stenoses enhances the speed of restenosis. However, Beathard [20] found similar results after the first, second, or third PTA. Uncontrolled and retrospective comparisons of the results of PTA of stenoses of thrombosed grafts *versus* those of non-thrombosed grafts suggest that outcome of the latter is somewhat better (reviewed in [21]). These studies suggest that outcome of treatment of less severe stenosis (not leading to thrombosis) is better than that of the more severe stenosis. Furthermore, studies suggest that secondary patency increases with access surveillance, repeated angiography, and stenosis correction as compared to an 'act only if thrombosed' approach [12,13]. Additionally, it is possible that overall morbidity and even mortality decreases. An elective treatment of stenosis is a less complicated procedure than thrombolysis combined with PTA. Furthermore, treatment of thrombosed grafts frequently necessitates placement of an intravenous catheter. Placement and use of these catheters is associated with considerable morbidity. All these issues need to be taken into account when balancing the cost-effectiveness of the introduction of periodic flow measurements.

Several other issues concerning flow measurements are still unclear. The optimal frequency of measurements and the optimal threshold level for intervention have not been determined. We found in a group of patients monitored by venous pressures that almost all thromboses that still occurred did so in patients with a flow <600 ml/min measured within the 2 months prior to thrombosis [19]. May *et al.* [8] reported that relative risk of thrombosis within 3 months after measurements was 1.36, 1.51 and 1.67 when flow was 850, 750 and 650 ml/min respectively. Venous pressure did not predict thrombosis. Sands *et al.* [22] found that patients with PTFE grafts and flow rates <800 ml/min had a 93% incidence of thrombosis during the 6 months following the measurements. These data seem to support the idea that by decreasing the frequency of flow assessment the cut-off value indicating increased risk of thrombosis increases.

This points to another deficiency in our knowledge. Basically, there are no data on the natural history of stenosis development and therefore on the change over time of risk of thrombosis. It seems likely that the absolute value of flow is related to the risk of thrombosis, whereas the decrease in flow over time reflects development of stenosis. Factors that influence the speed of development of stenosis are hardly known. It is conceivable that patients with high but decreasing flow need to be evaluated more frequently than patients with stable flow. In a recent study it was shown that especially a decrease in flow over time was predictive of imminent thrombosis [23].

There are now several devices that claim to provide accurate access flow measurements. Apart from the best validated device, i.e. the ultrasound dilution technique introduced by Krivitski (Transonic Hemodialysis Monitor, Transonic Systems Incorporated), a haematocrit dilution technique (Crit-Line Monitor, InLine Diagnostics) and a differential conductivity technique (Hemodynamic Monitor, Gambro) [24,25] are also available. The methods involve indicator dilution or conductivity tracer techniques. An indicator dilution technique detects recirculation by the dilution of arterial blood caused by a bolus of normal saline injected into the venous blood line; a conductivity tracer technique involves measurement of differential conductivity between arterial and venous blood flows after a bolus of hypertonic saline is injected into venous line as the conductivity 'tracer'. Both techniques are used while the patients dialysis blood lines are temporarily reversed to induce recirculation. From the measured recirculation and the knowledge of the dialyser blood flow rate, access blood flow can be calculated. Recently it was shown when compared to the ultrasound dilution technique that the technique based on differential conductivity measurements give virtually identical results for access flow, and that the Crit-Line device overestimates flow [24].

Recirculation

Access recirculation is defined as the return of dialysed blood to the arterial segment of the access bypassing the systemic circulation. This method was recently reviewed by Schneditz [26]. When compared with a method using a non-urea indicator, it became clear that in most cases values of $>10\%$ mean true access recirculation in most cases [27].

From a theoretical point of view it seems that recirculation occurs only when spontaneous graft flow approaches the level of dialyser blood flow, because in all other cases dialysed blood will not be allowed to

be taken up again by the arterial line of the extracorporeal circuit. Indeed, a recent study has indicated that recirculation is absent unless access blood flow is markedly impaired [28]. Therefore recirculation, when measured appropriately or by a non-urea method, can be taken at best as a crude and very late sign of access dysfunction.

Identifying native fistulae at risk of thrombosis

Once fully matured, thrombosis is a rare complication. Besarab *et al.* [13] has already noted that venous pressure monitoring was not useful in native accesses.

Recently it was argued that flow measurement in native fistulae poses problems [25], because needle placement is very critical. The arterial needle has to be placed in the main branch. Fistulae may have side-branches. Placement of the needles in two minor branches makes it impossible to measure flow.

Preliminary data by Sands *et al.* [29] also showed that monthly flow measurements in native fistulae did not result in a further reduction of the already very low thrombosis rate.

Conclusion

The concept that it is more important to recognize patients at risk of thrombosis, than identifying stenoses *per se*, is attractive from a theoretical point of view and is supported by clinical evidence. Improvement of current methods of identifying patients at risk of thrombosis early seems within reach. Recently introduced technology for flow measurement is promising. It may prove a worthwhile additive to present practice of access surveillance.

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Hepatitis C virus in the haemodialysis units: novel insights by new techniques?

Fabrizio Fabrizi and Francesco Locatelli

Nephrology and Dialysis Division, Lecco Hospital, Lecco, Italy

Introduction

Hepatitis C virus (HCV) infection is highly prevalent among patients on chronic haemodialysis (HD). This evidence was accumulated in early 1990s with an enormous body of data based on anti-HCV testing. However, anti-HCV assays have failed to unequivocally identify a protective antibody or an immune pattern. Furthermore, a large population of anti-HCV-positive patients do not show HCV viraemia.

The use of novel techniques for detecting viraemia and strains of HCV among HD patients has given us the opportunity to deepen our understanding of the virological and clinical features of HCV in HD. In fact, HD patients are a high-risk group for HCV; however, the immune compromise conferred from chronic uraemia may affect the biological properties of HCV in this population.

In this article, we report some recent advances in the biology of HCV in HD. HCV is an important agent of liver disease in chronic HD patients; the clinical implications of these new acquisitions may have an impact on the routine clinical activity of nephrologists within HD units.

Acquisition of HCV infection in HD

The directly quantitative branched-chain (bDNA) signal amplification assay provided information about the dynamics of HCV acquisition among patients on HD [1]. It is a signal-amplified oligonucleotide probe test [2] for detecting and quantifying HCV RNA in serum and avoids many of the pitfalls of reverse-transcription polymerase chain reaction (RT–PCR) technology. This assay is based on the specific hybridization of synthetic oligonucleotides (HCV capture probes and HCV extender probes) to the 5′ untranslated region and core genes of HCV RNA. It amplifies the reaction signal rather than the genome: advantages of the amplification include reproducible quantitation of results and elimination of false positives due to contamination. In addition, bDNA gives a direct quantitative result expressed as molecular equivalents of HCV RNA. It is slightly less sensitive than RT–PCR, however, it is as easy to perform as a microwell enzyme immunoassay (EIA) and may routinely provide a quantitative estimation of the genomic burden.

In some HD patients [1] we observed the same pattern of HCV acquisition: there was an initial viraemic phase associated with an increase in alanine transaminase (ALT) activity which preceded the anti-HCV seroconversion. This is followed by HCV RNA clearance and ALT normalization. Anti-HCV antibody appeared 1–2 months after the ALT increase. This pattern is compatible with a direct cytopathic effect of the virus; however, other explanations are equally plausible. A mild and short elevation in liver enzymes has been also observed about the time of HBsAg acquisition in chronic HD patients [3]. The peak of ALT during the initial phase of the HCV infection was not high (up to 74 IU/l); in a previous survey [4] some of the patients with de novo seroconversion for anti-HCV showed higher ALT levels (up to 341 IU/l). However, baseline values of aminotransferase activity are typically depressed in chronic HD patients [5–7].

This pattern of acquisition of HCV supports recent observations [7] that the relationship between detectable HCV RNA and raised aminotransferase values in serum is stronger than has so far been recognized, even if this may be masked by low AST and ALT baseline values. These results strongly confirm a prior recommendation [8] that serial ALT levels be monitored monthly in HD patients to detect subclinical liver disease and HCV acquisition. HCV RNA testing can identify HCV before seroconversion in individuals with deranged liver function tests. It is necessary to recognize that ALT levels in the 'normal' range for the general population may be indicative of a pathological state in HD; an increase in baseline level need not reach the 'abnormal' range to indicate the onset of acute HCV.

Viral load in HCV-infected patients on HD

A recent survey [9] in a large population of HD patients using bDNA assay reported that the viral load in HD is rather small (19.43×10^5 Eq/ml); the mean levels of HCV RNA in these patients are low compared with other patient groups with HCV, i.e. immunocompetent patients with acute ($1 \times 10^7 - 1 \times 10^9$ Eq/mL) [10] or chronic (8.4×10^6 Eq/ml) hepatitis C [11], haemophiliacs (2.8×106 Eq/ml) [12], liver trans-

Correspondence and offprint requests to: Fabrizio Fabrizi, MD, Divisione di Nefrologia e Dialisi, Azienda Ospedale di Lecco, via Ghislanzoni 22, I-23900 Lecco, Italy.

plant recipients (7.9×10^6 Eq/ml) [13] and renal transplant recipients (3.94×10^7 Eq/ml) [14] or individuals co-infected with human immunodeficiency virus (1.7×10^7 Eq/ml) [12]. Only health workers and intravenous drug users had HCV RNA levels (6.3×10^5 Eq/ml) slightly lower than those seen in HD patients [15]. It is probable that the infectivity of HCV in HD is also low, since an apparently non-linear relationship exists between viral titre and infectivity.

In addition, a multivariate analysis [9] showed an independent and significant association between anti-HCV antibody by ELISA 2.0 assay, ALT activity and HCV RNA values. The other demographic, clinical, biochemical and virological features of HD patients were not significantly associated with HCV viraemic burden.

Measurements using bDNA assay have recently been performed to provide insight into the course of the HCV load over time [16]. Different patterns of HCV viraemia have been detected: 'persistently' and 'intermittently' viraemic individuals, and persistently HCV RNA-negative patients on HD. In some cases the fluctuating course of HCV viraemia may be linked to low HCV RNA levels, i.e. below the cut-off value for the bDNA assay. Therefore, one determination of HCV RNA is not sufficient to accurately assess the virological status of anti-HCV-positive patients on chronic HD.

Fluctuations in the HCV viral load over time in either 'persistently' or 'intermittently' viraemic patients were low compared with the changes in HCV RNA in chronic HD patients after interferon treatment [17]. The HCV load was stable over a 13-month follow-up [16], the stability of HCV RNA over time could be related to the presence of a steady-state in these immunosuppressed individuals. The mechanisms of low and constant HCV RNA levels among HD patients, in spite of immunosuppression due to chronic uraemia are unclear, but the passage of HCV into the dialysate compartment [18] or the destruction of HCV during the passage across the dialyser membrane during HD [19] could play a role. Also, an increase in interferon activity promoted by the dialysis membrane has recently been reported [20]. The interplay between HCV and the immune system remains to be elucidated. It has even been suggested that the spectrum of host immune response to the virus in immunosuppressed patients is as variable as that seen in immunocompetent individuals [21]. Potential factors affecting progression include the degree of immune competence at the time of initial HCV infection and the dose of infectious virus, as well as intrinsic and extrinsic host-derived factors.

HCV infection in patients with normal immunological function tends to be indolent, but many HCV-infected patients develop cirrhosis or hepatocellular carcinoma [22]. Long observations are necessary to evaluate the course of the viral load in HD and to assess whether it is atypical in immunologically compromised patients on chronic HD treatment.

Epidemiological features of HCV in HD

HCV infection is endemic among patients on chronic HD. Use of the bDNA assay in a large population of HD patients [1] has confirmed the presence of a small but important group of HD patients with detectable HCV RNA in the serum who nevertheless are anti-HCV negative. It is likely that immunosuppression prevented the serological response to HCV in these patients. Therefore, serological surveys aimed at assessing HCV prevalence in HD units usually underestimate the exact frequency of HCV.

In addition, the failure of a significant number of HCV-infected dialysis individuals to produce antibody may affect the assessment of HCV incidence within dialysis units. In fact, de novo HCV infection in HD has recently been observed [23] in the absence of a serological response to HCV. Under these circumstances in our laboratory, we observed an initial viraemic phase associated with a rise in ALT into the 'abnormal range', followed by normalization of ALT. This pattern of HCV acquisition is also noted when bDNA testing is used [1]. Nevertheless in some cases de novo HCV infection was undetected by bDNA assay and anti-HCV. Only RT–PCR technology was able to detect HCV by direct measurement of HCV RNA [23]. Thus, RT–PCR technology should be incorporated into the diagnostic repertoire for HCV in HD patients. To exclude HCV infection in this population one has to use RT–PCR methodology.

Typing of HCV in HD

HCV has a high mutation rate and is present in nature as a population of different but closely related genomes [24]. Specific HCV genotypes [24] may be associated with different clinical manifestations, rates of disease progression and response to interferon treatment. Moreover, the routes and frequency of patient-to-patient transmission within dialysis units could be influenced by HCV genotypes. For these reasons, identification of the infecting HCV type may be very useful in the routine clinical activity of nephrologists within dialysis units. Precise assessment of the specific strain requires sequence analysis of the hypervariable region; however, this procedure is expensive, laborious and time-consuming. Alternatively, PCR with subtype-specific primers has been used to identify subtypes of HCV. Also PCR-based methods are cumbersome and unsuitable for analysing large cohorts of dialysis patients with HCV. Their applicability outside a research setting is limited. Recently, a novel assay for serological assessment of HCV types among patients with HCV has been developed [25]: the RIBA™ HCV serotyping strip immunoblot assay (SIA). It is based on RIBAs™ SIA methodology and is a highly reproducible and reliable technique for detecting HCV serotypes [25]. In RIBA™ HCV serotyping SIA, HCV peptides from the NS-4 and core regions of the HCV genome (Figure 1) are immobilized on a nitrocellulose

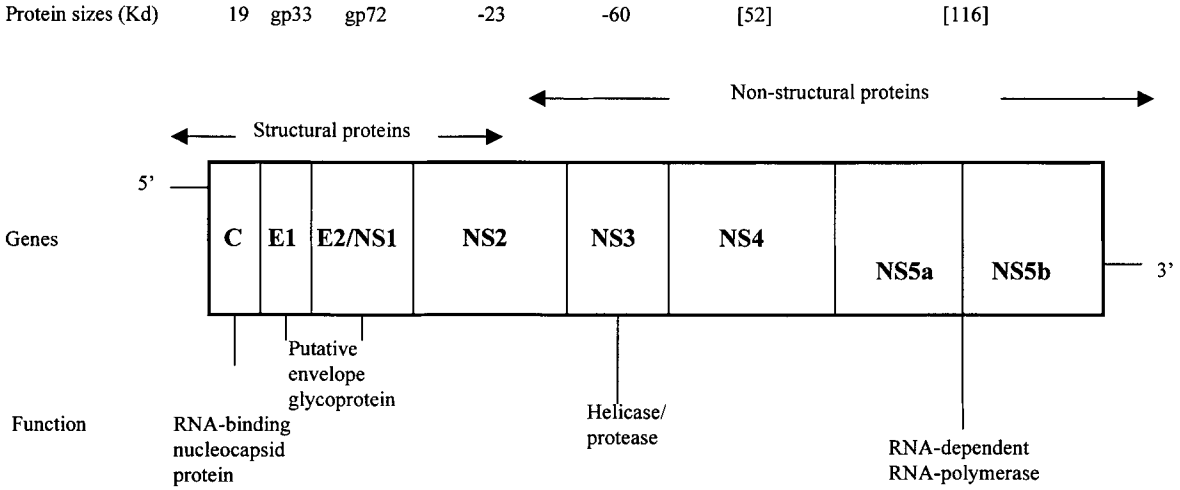

Fig. 1. Organization of the hepatitis C viral genome.

solid support where they may react with antibodies in the patient's serum. Antibodies to these specific HCV peptides bind to the RIBA℠ strip to create a dark band at the site of the IgG antibody-antigen complex. The reactivity present in the RIBA℠ strip is interpreted for assessment of HCV serotypes according to the algorithm reported elsewhere [25]. We used RIBA℠ HCV serotyping SIA to assess HCV types in a large population of HCV-infected dialysis patients and a comparison with RT–PCR was made [26]: the association between the results obtained by RIBA℠ HCV serotyping SIA with those found by RT–PCR technique was strong (93%). In contrast to the findings obtained among immunocompetent individuals, RIBA℠ HCV serotyping SIA was unable to detect any HCV reactivity in a subset (25%) of HD patients successfully genotyped [26]. The limited immunocompetence conferred by chronic uraemia prevented antibody production in these individuals.

Conclusions

The application of novel techniques such as RT–PCR, bDNA signal amplification assay and RIBA℠ HCV SIA shed light on some biological properties of HCV among HD patients. Using bDNA and RT–PCR technology, we observed a specific pattern of HCV acquisition in HD. The need to screen the HD population for ALT measurement combined with anti-HCV to control hepatitis C has been emphasized. However, there is circumstantial evidence showing that de novo HCV infection in HD may go undetected by serological tests and bDNA assay. The exclusion of HCV infection in the HD population requires RT–PCR technology. Assessment of HCV RNA by RT–PCR (or bDNA assay) is too expensive to be considered as the recommended screening test for HD patients; HCV RNA testing can identify HCV early if a suspicion of HCV exists in HD patients with deranged liver function tests. The infectivity of HCV among patients on HD is probably low as the viral load in this population is reduced and stable over time. Various patterns of viraemia in HCV-infected patients on HD exist: repeated testing for HCV RNA is necessary to accurately assess viraemia in this population. The HCV types in HD may be easily recognized by serological analysis. Chronic uraemia interferes with immunocompetence and this hampers serological response in a significant number of chronic HD patients with HCV.

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Ischaemic heart disease after renal transplantation: how to assess and minimize the risk

David C. Wheeler

Department of Nephrology, University Hospital NHS Trust, Birmingham, UK

Introduction

Advances in immunosuppressive therapy and in the treatment of opportunistic infection have greatly improved outcomes following renal transplantation, unmasking the clinical importance of co-morbid conditions associated with uraemia. It is now well established that patients with chronic renal failure are at increased risk of cardiovascular disease [1]. Long-term follow-up studies suggest that this problem is attenuated, but is not corrected, by successful renal transplantation even when biases resulting from patient selection are taken into account [2]. Cardiovascular disease has now emerged, ahead of infection, as the leading cause of mortality in renal transplant recipients, particularly after the first year [3]. Since many of these individuals die with functioning transplants, these deaths represent an increasingly important cause of graft loss. In one study from Scandinavia, more grafts were lost from patient mortality than rejection during the 2–5 year post-transplant period (49 *vs* 41%). Ischaemic heart disease and other vascular events collectively accounted for 63% of these deaths [4].

Efforts to extend patient and graft survival will therefore become increasingly dependent on appropriate prevention and management of cardiovascular disease, rather than on further advances in immunosuppressive or antimicrobial therapy. The following brief comments describe how screening strategies could be used to exclude patients with significant coronary

Correspondence and offprint requests to: Dr David C. Wheeler MD MRCP, Department of Nephrology, University Hospital NHS Trust, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, UK.

artery disease from transplant waiting lists and how the chances of developing disease might be minimized by appropriate risk factor modification. These approaches make sense when considering the allocation of cadaver kidneys, especially when organs are in short supply. Renal transplantation is generally cheaper than dialysis and death of a patient with a functioning kidney wastes the graft, thus negating the economic benefits of transplantation. Although not discussed, similar principles can be applied to detection and prevention of cerebrovascular, peripheral vascular, and left ventricular disease in patients considered for renal transplantation.

Assessing the risk of ischaemic heart disease

Perhaps not surprisingly, the presence of pre-transplant ischaemic heart disease has been shown to be a strong independent predictor of post-transplant cardiovascular events [5]. It therefore seems likely that operative risks could be minimized and graft survival improved if patients with established coronary disease were denied access to transplant waiting lists. In many cases, the presence of coexistent ischaemic heart disease will be clinically apparent. Guidelines drawn up by the American Society of Transplant Physicians recommend that individuals with angina pectoris, a history of myocardial infarction or congestive cardiac failure should undergo coronary angiography before any consideration is given to renal transplantation [6].

Many uraemic patients will have clinically silent disease. Since it is generally not practical to perform coronary angiography on all individuals considered for transplant listing, other approaches have been recommended. These include stratification of asymptomatic patients based on the level of risk (as discussed below) and the use of non-invasive tests to pre-select patients in whom more detailed investigation is appropriate [6]. Pre-selection should increase the utility of screening tests that are likely to be more effective in a population where the incidence of the disease is high.

Such an approach proved to be effective in a prospective study of 189 consecutive patients referred for transplantation. Those without risk factors received no further cardiac investigation, whilst those considered at risk on the basis of clinical characteristics underwent thallium myocardial scintigraphy. Over a mean follow-up period of 47 months, cardiac mortality was considerably higher in the latter group (17 *vs* 1%, $P<0.001$). The presence of reversible or fixed perfusion defects on thallium scans allowing further stratification of patients according to the risk of cardiac mortality [7].

With or without patient pre-selection, the ideal screening strategy for high-risk asymptomatic patients has not been established. Whilst the use of exercise electrocardiography, thallium scintigraphy and dopamine echocardiography have been reported, it is unclear whether these tests have sufficiently high positive and negative predictive values to allow accurate pre-selection of patients for coronary angiography [6]. Dopamine stress echocardiography looks the most promising with a reported sensitivity of 95% and specificity of 86% when compared to coronary angiography in a group of unselected patients with end-stage renal disease [8]. However, local expertise is likely to be an important factor in determining the success of a screening strategy and many renal units have developed their own protocols accordingly. Even when coronary angiograms are performed, the value of this test in predicting future acute coronary events in the context of chronic renal failure has not been established.

Minimizing the risk of ischaemic heart disease

The detection of coronary artery disease will not only deny some patients a place on the transplant waiting list, but will also identify those most likely to benefit from medical or surgical treatment. Both percutaneous transluminal coronary angiography (PTCA) and coronary artery bypass grafting (CABG) relieve symptoms of angina in patients with chronic renal failure. However, when compared to individuals without renal failure undergoing CABG, perioperative morbidity and mortality are increased as are restenosis rates following PTCA [9]. In a retrospective comparison of the two procedures, patients undergoing CABG were shown to have a lower incidence of recurrent angina, myocardial infarction and sudden cardiac death [10]. However, at the present time, there are no data confirming that such intervention improves survival in chronic renal failure. Thus it is unclear whether patients who have undergone revascularization procedures should subsequently be reconsidered for transplant listing.

Ischaemic heart disease remains a major cause of morbidity and mortality in the post-transplant period, even when efforts are made to exclude patients with pre-existing disease. In one follow-up study, 23% of patients who survived with a functioning graft for 15 years developed *de novo* coronary artery disease during this period [5]. It is therefore clear that preventative strategies are required to minimize the risks of ischaemic heart disease following renal transplantation.

Many risk factors for atherosclerosis can be identified in patients with chronic renal failure and may help to explain the markedly increased incidence of premature ischaemic heart disease in these individuals. These include smoking, hypertension, diabetes mellitus, dyslipidaemia, increased oxidant stress, elevated procoagulant activity, and hyperhomocysteinaemia [9]. However, to date there have been no prospective studies designed to demonstrate that modification of any risk factor will reduce the frequency of cardiovascular events, either pre- or post-transplantation. In the absence of these data, it is tempting to extrapolate from our knowledge based on the general population.

However, this approach should be cautious, since some studies have failed to demonstrate that commonly recognized risk factors such as hypertension and hypercholesterolaemia are independently associated with the development of coronary artery disease in the post-

transplant period [5]. Current recommendations aimed at minimizing the risk of cardiovascular disease complicating chronic renal failure emphasize cessation of smoking, avoidance of weight gain, dietary and lifestyle modification, optimization of diabetic control and treatment of hypertension and dyslipidaemia [9]. Strategies could also include dietary vitamin supplementation to reduce homocysteine levels and oxidant stress, antiplatelet drugs to decrease thrombogenic risk and correction of post-menopausal hormone deficiency. To maximize any potential benefits, risk factor management should ideally begin early in the course of renal disease, rather than in the post-transplant period. Benefits could include an increase in the proportion of patients suitable for transplant listing and improvements in the survival of individuals with chronic renal disease.

Conclusions

Ischaemic heart disease remains a major cause of morbidity and mortality in patients with chronic renal failure and markedly reduces life expectancy following renal transplantation. The detection, prevention and treatment of this and other cardiovascular diseases has become a management priority in individuals considered for renal transplantation. Future research should aim to establish the relationship between recognized risk factors and cardiovascular endpoints, the impact of risk factor modification on cardiovascular morbidity and mortality and whether active screening and revascularization programmes extend the lives of individuals with chronic renal failure, whether or not they are transplanted.

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