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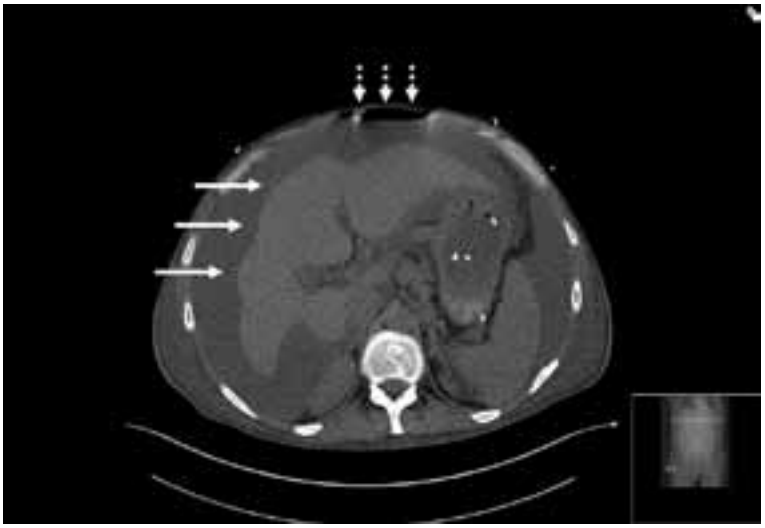

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EDITORIAL

Will laboratory markers replace kidney biopsy in patients with nephrotic syndrome?

J.J. Weening

University of Amsterdam, Amsterdam, Erasmus MC, Rotterdam, Tergooiziekenhuizen, the Netherlands,
e-mail: jweening@tergooiziekenhuizen.nl

Since its introduction in the 1950s,[1] the renal biopsy has become an important tool in the diagnosis and treatment of patients with kidney disease, allowing precise classification and a well-informed estimation of severity, duration and prognosis of the disease involved.[2,3]

In some patients with renal symptoms, a presumed diagnosis can be made without knowledge of the renal histology, e.g. in a young child with sudden onset nephrotic syndrome (probably minimal change nephropathy); in an adolescent with acute renal dysfunction one or two weeks after a streptococcal throat infection (post-infectious glomerulonephritis); or in a patient with intermittent gross haematuria with an otherwise normal renal function without proteinuria (thin basement membrane disease or uncomplicated IgA nephropathy).

In most patients with proteinuria with or without nephrotic syndrome, histology-based diagnosis of the underlying glomerular injury is important to guide treatment and allow a rational prognosis. The biopsy findings may be as diverse as diabetic nephropathy, amyoidosis due to plasma cell dyscrasia, autoimmune disease such as lupus erythematosus, or genetically dysregulated glomerular podocytes.

Also in patients with acute or rapidly progressive renal dysfunction, a renal biopsy is crucial: it may reveal widespread interstitial injury due to drug-induced allergic reactions or toxic cellular injury; acute or extracapillary glomerulonephritis related to infectious disease or an autoimmune reaction; or severe vascular injury in the context of systemic vasculitis or thrombotic microangiopathy. In transplant patients, knowledge of renal histopathology guides us through the confusing field of rejection, drug toxicity, viral and other infections, recurrence of the original injury or development of a *de novo* form of renal disease.

Notwithstanding the important contribution of the renal biopsy for immediate and unequivocal diagnosis in most patients with kidney disease, other diagnostic tools may be helpful in postponing the procedure, allowing for an early start of treatment. Patients who enter the hospital with rapidly evolving renal failure, signs of glomerular disease such as proteinuria and erythrocyturia and a high *anti-neutrophil cytoplasmic antibody (ANCA) antigen titre,* may be started on cytotoxic and immunosuppressive treatment while awaiting a renal biopsy. A subsequent biopsy provides the physician with precise information of the extent and severity of renal injury. This is helpful in providing reassurance for the highly toxic treatment and gives a better insight into how much recovery can be hoped for.[4]

Similarly, in a patient with high titres of anti-DNA autoantibodies, multisystem signs of systemic lupus erythematosus (SLE) and symptoms of renal involvement with loss of renal function, proteinuria and haematuria, a renal biopsy is not decisive in making the diagnosis of active SLE, and therefore treatment can be started without delay. However, a biopsy is still necessary for classifying the type of renal disease in this patient and for estimation of activity and chronicity of the changes, allowing appropriate treatment choices.[5]

In membranous nephropathy (MN), the most frequent form of proteinuric renal disease in adults, the renal biopsy has always been central in distinguishing it from other causes of nephrotic syndrome such as minimal change glomerulopathy, focal and segmental glomerulosclerosis, diabetic nephropathy, amyloidosis and light chain disease. MN can be secondary to infectious disease, malignancy and systemic lupus, but in most cases it is idiopathic. Until three years ago, the underlying cause of the formation of subepithelial immune deposits in the glomerular capillary wall in idiopathic MN was unknown, although experimental work had long hinted at an autoimmune mechanism involving one or more podocyte proteins as antigen targets.[6] In 2009, the enigma was solved by Beck *et al.*,[7] who demonstrated that an autoantibody response to phospholipase A2-receptor causes the disease in 75%

of cases of MN – the other cases being secondary to e.g. infectious diseases, malignancy or systemic autoimmune disease. Further studies revealed the underlying genetic susceptibility, based on polymorphisms in the PLA2R and HLA-DQ genes that act together in the development of this autoimmune disease.[8] Several diagnostic tests are being developed to detect circulating anti-PLA2R autoantibodies in serum samples, which will be important for diagnosis, evaluation of therapeutic intervention, and follow-up in patients with native renal disease and after transplantation. In the perspective of this new exciting knowledge, in the current issue of our journal Hofstra and Wetzels[9] discuss the clinical value of the currently available serum test and the renal biopsy in the nephrotic patient who tests positive for circulating anti-PLA2R antibodies. In their view it is still too early to go without renal histopathology and to rely on the serum test only, because most studies so far have been retrospective and more robust tests should be developed allowing quantification. Different tests should be compared and specificity and sensitivity of these tests to identify idiopathic MN have to be established in prospective studies. Furthermore, there are still several questions to be answered regarding the pathophysiology of the PLA2R-binding antibodies and the role of Ig isotypes and complement.

Finally, possible concomitant diseases may be missed when a biopsy is omitted and there is an additional need for estimating the evolution of the lesion over time (MN is usually classified in four stages from early to late) and the extent of chronic damage such as glomerulosclerosis and interstitial fibrosis, since these pathological changes may have an important effect on prognosis and the need for further treatment.

The exciting discovery in the field of idiopathic MN has solved many of the questions related to the cause and pathogenesis of this renal disease and holds great promise for the patient, but it seems too soon after the seminal observation of Beck and colleagues to rely on a preliminary test in order to distinguish between idiopathic and secondary forms of MN and to discard the cornerstone of its diagnosis: the renal biopsy.

REFERENCES

1. Muehrcke RC, Kark RM, Pirani CL. Biopsy of the kidney in the diagnosis and management of renal disease. N Engl J Med. 1955;253:537-46.
2. Turner MW, Hutchinson TA, Berre PE, et al. A prospective study on the impact of the renal biopsy in clinical management. Clin Nephrol. 1986;26:217-21.
3. Madaio MP. Renal Biopsy. Kidney Int. 1990;38:529-32.
4. Berden AE, Ferrario F, Hagen EC, et al. Histopathological classification of ANCA-associated glomerulonephritis. J Am Soc Nephrol. 2010;21:1628-36.
5. Weening JJ, D'Agati VD, Schwartz, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. Kidney Int. 2004;65:521-30.
6. Van Damme BJC, Fleuren GJ, Bakker WW et al.) Experimental glomerulonephritis in the rat induced by antibodies to tubular antigens. V. Fixed glomerular antigens in the pathogenesis of heterologous immune complex glomerulonephritis. Lab Invest. 1978;38:502-10.
7. Beck LH, Bonegio RGB, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med. 2009;361:11-21.
8. Stanescu HC, Arcos-Burgos M, Medlar A, et al. Risk HLA-DQA1 and PLA(2)R1 alleles in idiopathic membranous nephropathy. N Engl J Med. 2011;364:616-26.
9. Hofstra J, Wetzels JF. Anti-PLA2R antibodies in membranous nephropathy: ready for routine clinical practice? Neth J Med. 2012;70:109-13.

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REVIEW

Anti-PLA_2R antibodies in membranous nephropathy: ready for routine clinical practice?

J.M. Hofstra*, J.F. Wetzels

Department of Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands,
*corresponding author: tel.:+ 31 (0)24-3614761, fax: +31 (0)24-3540022, e-mail: J.Hofstra@nier.umcn.nl

ABSTRACT

The identification of circulating autoantibodies against the M-type phospholipase A_2 receptor (anti-PLA_2R) in patients with idiopathic membranous nephropathy (iMN) has been a major discovery. Anti-PLA_2R can be measured by a commercially available test. It is suggested that measurement of anti-PLA_2R will change the diagnostic strategy in patients with nephrotic syndrome and may guide treatment in patients with iMN. We review the available evidence and caution against the immediate injudicious use of the assay in routine clinical practice.

KEYWORDS

Membranous nephropathy, anti-PLA_2R, nephrotic syndrome

INTRODUCTION

Idiopathic membranous nephropathy (iMN) is the most common cause of nephrotic syndrome in the adult Caucasian population.[1] It is well established that iMN can develop due to the binding of a circulating antibody to an antigen that is present on podocytes.[2] In 2009 Beck *et al*. identified the M-type phospholipase A_2 receptor as an important antigenic target.[3] The authors showed that PLA_2R is expressed on podocytes and that antibodies against native PLA_2R, primarily of the IgG4 subclass, were present in the serum of approximately 70% of patients with iMN. This study provided the evidence that iMN is an autoimmune disease. The important role of PLA_2R in the pathogenesis was supported by the highly significant association between single nucleotide polymorphisms in the PLA_2R gene and the development of iMN.[4]

Membranous nephropathy can also develop secondary to systemic autoimmune diseases (SLE), infections (hepatitis B), drugs (NSAIDs), and malignancies. In these conditions the subepithelial deposits may arise from deposition of circulating immune complexes in the capillary wall or from binding of antibodies to antigens that are derived from the tumour and were planted in the basement membrane.[5]

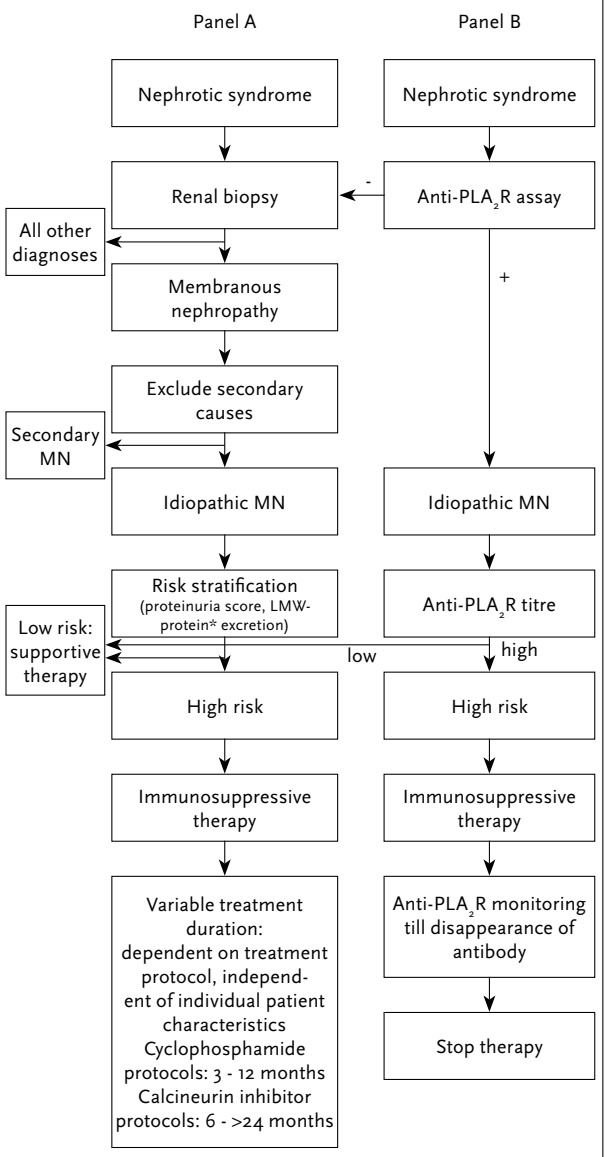
Beck *et al*. already suggested that the detection of anti-PLA_2R in a patient with nephrotic syndrome may be pathognomonic for idiopathic MN, thus obviating the need for a diagnostic renal biopsy and an extensive search for underlying causes. Their findings and other recent data predicted that measurement of anti-PLA_2R may change the diagnostic algorithm in patients with nephrotic syndrome and guide treatment decisions in patients with iMN (*figure 1*).

Measurement of anti-PLA_2R is now possible with the development of an easy to use, commercially available assay. We briefly review the current evidence and express our view on the usefulness of this assay now and in the near future.

MEASUREMENT OF ANTI-PLA_2R ANTIBODIES: THE TECHNIQUES

Beck *et al*. employed a Western blot technique using glomerular extracts which were electrophoresed under nonreducing conditions.[3] Human serum was used as primary antibody, sheep antihuman IgG subclass antibodies as secondary antibodies, and finally peroxidase-labelled antisheep antibodies were used for detection. Quantitation was done by densitometric analysis and values expressed in arbitrary units. More recently an indirect immunofluorescence technique (IFT) was developed, which is now commercially available.[6] For this assay slides are made that contain biochips containing HEK 293 cells transfected with cDNA coding for PLA_2R and non-transfected cells as control. The biochips are incubated with human serum in different dilutions. The bound antibodies are detected

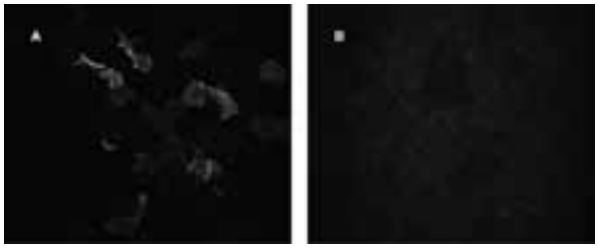
Figure 1. *Anticipated role of anti-PLA$_2$R antibody assay in the diagnosis and treatment of patients with nephrotic syndrome and membranous nephropathy*



Panel A illustrates the current algorithm for the diagnosis and treatment of idiopathic membranous nephropathy (adapted from Hofstra).[19] Panel B illustrates future practice if the ongoing prospective studies confirm the high specificity of anti-PLA2R for the diagnosis of idiopathic membranous nephropathy. We have also depicted the potential role of a quantitative assay in predicting risk of progression and determining duration of therapy. LMW-protein = low-molecular-weight protein; MN = membranous nephropathy.

with FITC-labelled goat antihuman IgG. The test result in an anti-PLA$_2$R-positive patient is depicted in *figure 2*. Quantitation can be performed by using different serum dilutions. UK investigators have developed an ELISA assay, which allows rapid and simple quantitation, but is only used for research purposes.[7] It is expected that commercial ELISA assays will become available in 2012. With such assays, quantitation will become simple and cost effective. Preliminary data suggest that there are large discrepancies between the quantitative results of the IFT and ELISA assays.

Figure 2. *Indirect immunofluorescence test for anti-PLA$_2$R antibodies*



A: In a positive patient the antibodies bind to the PLA2R-positive HEK293 cells, leading to a positive immunofluorescence staining. B: The non-transfected PLA$_2$R-negative HEK293 cells serve as a control.

ANTI-PLA$_2$R IN IDIOPATHIC MEMBRANOUS NEPHROPATHY

In the pivotal study by Beck and coworkers, anti-PLA$_2$R antibodies were found in 70% of patients with iMN. Meanwhile, several studies have reported the prevalence of anti-PLA$_2$R-positive patients in different cohorts. These data are summarised in *table 1*. The percentage of anti-PLA$_2$R-positive patients ranges from 52% in a German study to 82% in a Chinese cohort.[6,8] These differences may be explained by differences in race, in the technique of the assay, or in the clinical characteristics. The study by Hoxha *et al.*, which reported the prevalence of 52%, was a cross-sectional study. These authors included patients with active as well as inactive disease. In 48% of patients no data on proteinuria were available. When limiting the analysis to patients with proteinuria >3.5 g/day, which likely reflects active disease, the percentage of patients with anti-PLA$_2$R antibodies was 66%. The Chinese group of Qin *et al.*, who reported an initial prevalence of 82%, repeated their assay in the negative patients, using less diluted patient serum and a higher concentration of detecting antibody. They observed a low titre of anti-PLA$_2$R antibody in 10 of 11 apparently negative patients. This study suggests that almost all patients with iMN may have detectable serum antibodies against PLA$_2$R.

ANTI-PLA$_2$R IN SECONDARY MEMBRANOUS NEPHROPATHY

Several authors have measured the presence of anti-PLA$_2$R antibodies in patients with secondary MN (*table 2*). It is obvious that the number of patients with secondary MN

Table 1. *Prevalence of anti-PLA$_2$R in idiopathic membranous nephropathy*

Author (year)	Patients (n)	aPLA$_2$R + (n; %)	Assay	Remarks
Beck (2009)[3]	37	26 (70)	WB	
Hofstra (2011)[17]	18	14 (78)	WB	
Beck (2011)[18]	35	25 (71)	WB	
Debiec (2011)[10]	42	24 (57)	IFT	
Hoxha (2011)[6]	100	52 (52)	IFT	Cross-sectional study; 66% positive if limiting analysis to patients with proteinuria >3.5 g/day
Qin (2011)[8]	60	49 (82)	WB	Low titres of PLA$_2$R present in 10/11 patients who were negative in standard assay Renal biopsies with mesangial or subendothelial deposits or glomerular infiltrating cells were excluded
Bruschi (2011)[20]	24	14 (58)	WB	Patient characteristics not provided
Beck (2011)[21]	14	12 (86)	WB	
Hoxha (2011)[11]	81	53 (65)	IFT	Prospective study
Schönermarck (2011)[9]	16	11 (69)	IFT	
Kanigicherla (2011)[7]	40	29 (73)	ELISA	Cross-sectional study; table reflects only patients with active disease

aPLA$_2$R + = anti-PLA$_2$R antibodies detectable; WB = Western blot; IFT = immunofluorescence test; ELISA = enzyme-linked immunosorbent assay.

included in the various studies is very low. Although most patients with secondary MN were negative for anti-PLA$_2$R antibodies, conflicting results have been reported. Of note, the authors suggest that the presence of anti-PLA$_2$R antibodies in patients with secondary MN might result from the co-incidental simultaneous development of iMN and the systemic disease, such as sarcoidosis or a malignancy. This suggestion was supported by Qin *et al.* who showed that in the PLA$_2$R-positive patients proteinuria persisted despite resection of the tumour.[8] These authors also showed that PLA$_2$R positivity paralleled the presence of IgG4 in the biopsy.

However, it is evident that more data are needed before we can safely conclude that the presence of anti-PLA$_2$R antibodies always reflects iMN and obviates the need to search for an underlying cause.

ANTI-PLA$_2$R IN PATIENTS WITHOUT MEMBRANOUS NEPHROPATHY

PLA$_2$R antibodies have not been detected in healthy controls (*table 3*). The antibodies were also absent in patients with proteinuria due to other glomerular diseases such as minimal change nephropathy, focal segmental glomerulosclerosis, or IgA nephropathy. However, the numbers in the literature are small. The total number of patients with non-membranous glomerular disease was 15 in Beck's study and 14 in a recent study by Schönermarck.[3,9] Hoxha studied 90 patients; however, only 18 had proteinuria >3.5 g/day.[6]

ANTI-PLA$_2$R ANTIBODIES IN MEMBRANOUS NEPHROPATHY: DISCORDANCE BETWEEN SERUM AND BIOPSY DATA

The discovery of anti-PLA$_2$R antibodies in serum of patients with MN and the presence of PLA$_2$R on the podocyte seemed to have solved the pathogenesis of MN. However, thus far there is no proof that the antibodies are pathogenic. There are no experimental models that are suited to study the pathogenesis of MN and the role of anti-PLA$_2$R since PLA$_2$R is not expressed in rat or mouse glomeruli. To study the pathogenicity of anti-PLA$_2$R antibodies the development of mice that stably overexpress PLA$_2$R in podocytes is required.

Recent studies further questioned the role of serum anti-PLA$_2$R as the main pathogenic antibody in iMN. Debiec *et al.* measured anti-PLA$_2$R in the serum of 42 patients with iMN and assessed the presence of

Table 2. *Prevalence of anti-PLA$_2$R antibodies in secondary membranous nephropathy*

Author	SLE		HBV/HCV		Malignancy		Other	
	N	aPLA$_2$R+	N	aPLA$_2$R+	N	aPLA$_2$R+	n	aPLA$_2$R+
Qin[8]	20	1	16	1	10	3	-	-
Hoxha[6]	6	0	1	0	3	0	7	0
Beck[3]	6	0	2	0	-	-	-	-
Knehtl[22]	-	-	-	-	-	-	1	1
Brenchley#	20	1			6	1		

SLE = systemic lupus erythematosus; HBV = hepatitis B virus; HCV = hepatitis C virus; aPLA$_2$R+ = anti-PLA$_2$R antibodies detectable; # personal communication ASN Kidney week 2011.

Table 3. *Prevalence of anti-PLA$_2$R antibodies in non-membranous nephropathy*

Author	Controls		Patients with glomerular disease		Remarks
	n	aPLA$_2$R+	n	aPLA$_2$R+	
Beck[3]	30	0	15	0	DN and FSGS
Qin[8]	20	0	-	-	
Hoxha[6#]	153	0	18	0	Most MCD n=10
Schönermarck[9]	-	-	14	0	Unknown

aPLA$_2$R + = anti-PLA$_2$R antibodies detectable; DN = diabetic nephropathy; FSGS = focal segmental glomerulosclerosis; MCD = minimal change disease; # analysis restricted to patients with proteinuria >3.5 g/day.

PLA$_2$R antigen in the renal biopsies.[10] Although there was concordance in the majority of patients, important exceptions were noted. In ten patients serum was negative whereas the renal biopsy was positive; the opposite occurred in three other patients. Hoxha also studied serum and biopsies in parallel.[11] PLA$_2$R expression was seen in renal biopsies of 47 patients, antibodies were found in 45 of them. In contrast, these authors did not report patients with detectable antibodies in serum, without expression of the antigen in the biopsy.

It is well know that iMN is an IgG4 dominant disease, with IgG4 being the dominant IgG subclass in renal biopsies of patients with iMN. Both Beck *et al.* and Qin *et al.* showed that antibodies against PLA$_2$R were mainly of the IgG4 subclass, confirming the dominance of IgG4.[3,8] Since IgG4 is not binding complement, this has sparked the debate on the pathogenic role of these antibodies. In a recent study published in abstract form it was suggested that PLA$_2$R IgG4 may bind mannose binding lectin (MBL), and thus activates complement via the MBL pathway.[12]

However, both Beck and Qin agree that other subclass specificities, particularly of the IgG1 and IgG3 subclass, can be found in most patients. The role of IgG4 is further questioned by the finding that IgG4 deposits in the biopsy do not always correlate with anti-PLA$_2$R titre in serum. Qu *et al.* did not observe IgG4 in 6/42 patients with iMN.[13] Hoxha reported three patients with anti-PLA$_2$R in serum and PLA$_2$R in the biopsy with negative IgG4 staining.[11]

ANTI-PLA$_2$R AND RECURRENCE OF IMN AFTER TRANSPLANTATION

In patients with iMN who develop end-stage renal disease, renal transplantation is the preferred therapy. Unfortunately, the post-transplant course is complicated by a recurrence of the disease in 10 to 45% of patients.[14]

The discovery of anti-PLA$_2$R sparked studies that evaluated the role of these antibodies in developing and predicting recurrent disease. In a recent case report Stahl *et al.* described a patient with high anti-PLA$_2$R titres at the time of transplantation, who developed a recurrence almost immediately after transplantation.[15]

The potential role of anti-PLA$_2$R antibodies in recurrent membranous nephropathy was questioned by Debiec *et al.*[16] These authors reported ten kidney transplant recipients with iMN and recurrent disease and six patients with iMN without recurrence after transplantation. In six out of ten patients with recurrent disease PLA$_2$R was present in deposits in the native kidney biopsy, suggesting anti-PLA$_2$R-related disease. In five of those, anti-PLA$_2$R antibodies were detectable in serum and/or biopsy at the time of recurrence. Of note, in one patient there was recurrence in the graft, but anti-PLA$_2$R antibodies were not detectable. Moreover, the course was peculiar in two other patients. In one patient, serum antibodies were present at the time of transplantation. The anti-PLA$_2$R had disappeared at the time of recurrence, although the biopsy expressed PLA$_2$R in the deposits. The second patient had anti-PLA$_2$R antibodies in the serum, but no PLA$_2$R detectable in the biopsy.

On the other hand, in three out of six patients without recurrence, antibodies were present in the serum at the time of transplantation. Apparently, the presence of anti-PLA$_2$R antibodies did not lead to recurrent disease in these patients up till four years after transplantation. Clearly, more data are needed before firm conclusions can be made.

ANTI-PLA$_2$R AND TREATMENT OF MEMBRANOUS NEPHROPATHY

Beck *et al.* described the association between the presence of circulating anti-PLA$_2$R and clinical disease activity in eight patients with iMN.[3] In a subsequent collaborative study we studied 18 patients with nephrotic syndrome.[17] In 14 patients antibodies against PLA$_2$R were found. Antibody status was determined in serum samples obtained at baseline, during remission and during relapse. We observed a striking correlation between anti-PLA$_2$R titre and proteinuria, when using both baseline data and data from all time points. Moreover, anti-PLA$_2$R antibodies disappeared in all patients but one during remission, and reappeared in all evaluable patients during relapse. These studies were extended by the Mayo group.[18] These investigators evaluated the time course of anti-PLA$_2$R titres in relation to proteinuria and outcome in patients with iMN treated with rituximab. They observed that the decrease of anti-PLA$_2$R titre preceded the decrease of proteinuria, and predicted outcome.

Kanigichirla *et al.* measured anti-PLA_2R by ELISA. In their cross-sectional analysis antibody positivity was related to disease activity.[7] Antibodies were present in 29 of 40 patients with active disease (73%), compared with 15% positivity in patients in partial or complete remission. Moreover, the authors noted that in patients with active disease the titre of PLA_2R predicted outcome.

An association between anti-PLA_2R titres and outcome was also suggested by Qin *et al.* In patients with low titres time to remission was considerably shorter (6.6 *vs* 14.5 months), and likelihood of remission was higher (50 *vs* 30% at 12 months).[8]

CONCLUSIONS

The discovery of anti-PLA_2R antibodies in patients with idiopathic membranous nephropathy is of utmost importance. This finding has established iMN as an autoimmune disease and greatly stimulated research on its pathogenesis. It is expected that the measurement of anti-PLA_2R antibodies will improve our diagnostic and therapeutic strategy in patients with nephrotic syndrome in general and iMN in particular. However, there are some caveats. Conclusions so far are based on small, mostly retrospective studies. The accuracy of the test to identify iMN and to exclude secondary causes of MN awaits well-designed prospective studies, which are currently underway. We do not know if assays are comparable. It is expected that quantitation of the anti-PLA_2R antibodies will be important for prediction of prognosis as well as guidance of therapy. In this respect we are eagerly awaiting the development of an ELISA assay.

In our opinion it is too early to discard a renal biopsy in patients with nephrotic syndrome. Also, we advise to carefully check secondary causes using simple tools in all patients with MN.[19] Meanwhile, we advise to store serum samples taken at baseline and during follow-up. This would allow to perform measurements at a time point when questions regarding therapy efficacy or cause of relapsing proteinuria arise.

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REFERENCES

1. Tiebosch AT, Wolters J, Frederik PF, et al. Epidemiology of idiopathic glomerular disease: a prospective study. Kidney Int. 1987;32(1):112-6.
2. Debiec H, Ronco P. Fetomaternal alloimmunization with antenatal glomerulopathies. Ann N Y Acad Sci. 2007;1110:559-66.
3. Beck LH, Bonegio RG, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med. 2009;361(1):11-21.
4. Stanescu HC, Arcos-Burgos M, Medlar A, et al. Risk HLA-DQA1 and PLA(2)R1 alleles in idiopathic membranous nephropathy. N Engl J Med. 2011; 364(7):616-26.
5. Glassock RJ. Human idiopathic membranous nephropathy--a mystery solved? N Engl J Med. 2009;361(1):81-3.
6. Hoxha E, Harendza S, Zahner G, et al. An immunofluorescence test for phospholipase-A-receptor antibodies and its clinical usefulness in patients with membranous glomerulonephritis. Nephrol Dial Transplant. 2011;26(8):2526-32.
7. Kanigicherla DKA, Brenchley PE, Venning M, et al. Association of Anti-PLA2R with Disease Activity and Outcome in Idiopathic Membranous Nephropathy [Abstract]. J Am Soc Nephrol. 2011;22:33A.
8. Qin W, Beck LH, Jr., Zeng C, et al. Anti-phospholipase A2 receptor antibody in membranous nephropathy. J Am Soc Nephrol. 2011;22(6):1137-43.
9. Schönermarck U, Eichhorn P, Sitter T, et al. Performance of Anti-Phospholipase A2-Receptor Antibody Testing for Membranous Nephropathy in Routine Clinical Practice [Abstract]. J Am Soc Nephrol. 2011;22:775A.
10. Debiec H, Ronco P. PLA2R autoantibodies and PLA2R glomerular deposits in membranous nephropathy. N Engl J Med. 2011;364(7):689-90.
11. Hoxha E, Zahner G, Velden J, et al. Phospholipase A2 Receptor Autoantibodies (PLA2R-AB) and Increased PLA2R Expression in Glomeruli Discriminate Primary from Secondary Membranous Nephropathy (MN) [Abstract]. J Am Soc Nephrol. 2011;22:68A.
12. Ma H, Beck LH, Salant DJ. Membranous Nephropathy-Associated Anti-Phospholipase A2 Receptor IgG4 Autoantibodies Activate the Lectin Complement Pathway [Abstract]. J Am Soc Nephrol. 2011;22:62A.
13. Qu Z, Liu G, Li J, et al. Absence of glomerular IgG4 deposition in patients with membranous nephropathy may indicate malignancy. Nephrol Dial Transplant 2011; doi: 10.1093/ndt/gfr534.
14. Choy BY, Chan TM, Lai KN. Recurrent glomerulonephritis after kidney transplantation. Am J Transplant. 2006;6(11):2535-42.
15. Stahl R, Hoxha E, Fechner K. PLA2R autoantibodies and recurrent membranous nephropathy after transplantation. N Engl J Med. 2010;363(5):496-8.
16. Debiec H, Martin L, Jouanneau C, et al. Autoantibodies specific for the phospholipase A2 receptor in recurrent and De Novo membranous nephropathy. Am J Transplant. 2011;11(10):2144-52.
17. Hofstra JM, Beck LH, Beck DM, et al. Anti-phospholipase A receptor antibodies correlate with clinical status in idiopathic membranous nephropathy. Clin J Am Soc Nephrol. 2011;6(6):1286-91.
18. Beck LH, Fervenza FC, Beck DM, et al. Rituximab-induced depletion of anti-PLA2R autoantibodies predicts response in membranous nephropathy. J Am Soc Nephrol. 2011;22(8):1543-50.
19. Hofstra JM, Wetzels JF. Management of patients with membranous nephropathy. Nephrol Dial Transplant. 2011; doi: 10.1093/ndt/gfr371.
20. Bruschi M, Carnevali ML, Murtas C, et al. Direct characterization of target podocyte antigens and auto-antibodies in human membranous glomerulonephritis: Alfa-enolase and borderline antigens. J Proteomics. 2011 Sep 6;74(10):2008-17.
21. Beck LH, Jr., Fervenza FC, Bomback AS, et al. Response of Anti-PLA2R to Adrenocorticotropic Hormone (ACTH) Gel in Membranous Nephropathy [Abstract]. J Am Soc Nephrol. 2011;22:33A.
22. Knehtl M, Debiec H, Kamgang P, et al. A case of phospholipase A receptor-positive membranous nephropathy preceding sarcoid-associated granulomatous tubulointerstitial nephritis. Am J Kidney Dis. 2011;57(1):140-3.

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REVIEW

New insights into pathways that determine the link between infection and thrombosis

M. Levi[1]*, T. van der Poll[1,2], M. Schultz[3,4]

[1]Department of Medicine, [2]Laboratory of Experimental Medicine and Center for Infection and Immunity Amsterdam (CINIMA), [3]Laboratory for Experimental Intensive Care and Anesthesiology, [4]Department of Intensive Care, Academic Medical Center, University of Amsterdam, the Netherlands, *corresponding author: e-mail: m.m.levi@amc.uva.nl

ABSTRACT

Severe infection is often linked to prothrombotic events. Indeed, haemostatic abnormalities are encountered in most cases of infection, ranging from an increase in sensitive markers for coagulation activation or insignificant laboratory changes to gross activation of coagulation that may result in localised thrombotic complications or disseminated intravascular coagulation. Systemic inflammation as a consequence of infection results in activation of coagulation, due to tissue factor-mediated thrombin generation, down-regulation of physiological anticoagulant mechanisms, and inhibition of fibrinolysis. Pro-inflammatory cytokines, immune cells and the endothelium form the interface on which differential effects on the coagulation and fibrinolysis pathways may ensue. Conversely, activation of the coagulation system may importantly affect inflammatory responses by direct and indirect mechanisms. Apart from the general coagulation response to inflammation associated with severe infection, specific infections may cause distinct features, such as haemorrhagic fever or thrombotic microangiopathy.

KEYWORDS

Infection, inflammation, thrombosis, coagulation, endothelium, cytokines

INTRODUCTION

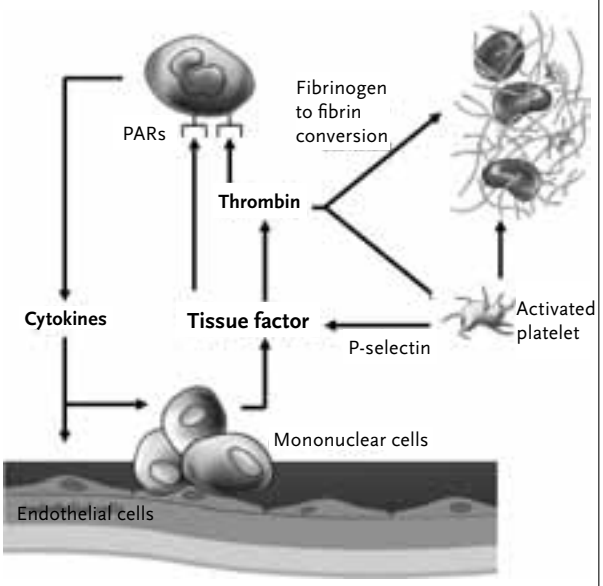
Increasing evidence points to a tight interaction between coagulation on the one hand and inflammation as a response to severe infection or chronic inflammatory states on the other hand.[1-3] In recent years the various mechanisms that play an important role in this interaction have been elucidated and this knowledge has indeed been demonstrated to be applicable for the improvement of our understanding of the pathogenesis of severe infection or chronic inflammatory states and, even more importantly, the clinical management of these patients.[4,5] In this article the mechanisms that play a role in the interaction between infection, inflammation and coagulation will be reviewed. Specific features of infectious disease-mediated effects on the coagulation system will be highlighted and the relevance for clinically relevant thrombotic manifestations is discussed.

INFECTION AND INFLAMMATION RESULT IN ACTIVATION OF COAGULATION

Acute inflammation, as a response to severe infection or trauma, results in a systemic activation of the coagulation system.[4,6] It was initially thought that this systemic activation of coagulation was a result of direct activation of the contact system of coagulation by microorganisms or endotoxin. However, in the 1990s it became apparent that cytokines played a mediatory role in the activation of coagulation and subsequent fibrin deposition and that the point of impact on the coagulation system was rather the tissue factor-factor VIIa ('extrinsic') pathway than the contact system ('intrinsic pathway').[7,8] Furthermore, the significance of impaired physiological anticoagulant pathways became increasingly clear.[9] Lastly, it was shown that impaired fibrin removal by a suppressed fibrinolytic system contributed importantly to the microvascular deposition of fibrin.

Vascular endothelial cells play a central role in all mechanisms that contribute to inflammation-induced activation of coagulation (*figure 1*). Endothelial cells respond

Figure 1. *Schematic representation of the link between infection/inflammation and coagulation*



Activated mononuclear cells and endothelial cells induce expression of tissue factor that activates platelets and the coagulation system. Activated coagulation proteases bind to protease-activated receptors (PARs), which may induce additional pro-inflammatory stimuli by releasing cytokines that target endothelial cells and mononuclear cells.

to the cytokines expressed and released by activated leucocytes but can also release cytokines themselves.[10] Furthermore, endothelial cells are able to express adhesion molecules and growth factors that may not only promote the inflammatory response further but also affect the coagulation response. However, it has recently become clear that, in addition to these mostly indirect effects of the endothelium, endothelial cells interfere directly with the initiation and regulation of fibrin formation and removal during severe infection.[11,12]

MECHANISMS OF THE INFECTION-INDUCED ACTIVATION OF COAGULATION

Inflammation-induced coagulation activation is characterised by widespread intravascular fibrin deposition, which appears to be a result of enhanced fibrin formation and impaired fibrin degradation.[1,13] Enhanced fibrin formation is caused by tissue factor-mediated thrombin generation and simultaneously occurring depression of inhibitory mechanisms, such as the protein C and S system. The impairment of endogenous thrombolysis is mainly due to high circulating levels of plasminogen activator inhibitor type 1 (PAI-1), the principal inhibitor of plasminogen activation. These derangements in coagulation and fibrinolysis are mediated by differential effects of various pro-inflammatory cytokines.[7]

Tissue factor plays a central role in the initiation of inflammation-induced coagulation.[14] Blocking tissue factor activity completely inhibits inflammation-induced thrombin generation in models of experimental endotoxaemia or bacteraemia.[15,16] The vast majority of cells constitutively expressing tissue factor are found in tissues not in direct contact with blood, such as the adventitial layer of larger blood vessels. However, tissue factor comes into contact with blood when the integrity of the vessel wall is disrupted or when endothelial cells and/or circulating blood cells start expressing tissue factor. The *in vivo* expression of tissue factor seems mostly dependent on interleukin (IL)-6, as demonstrated in studies showing that inhibition of IL-6 completely abrogates tissue factor-dependent thrombin generation in experimental endotoxaemia, whereas specific inhibition of other pro-inflammatory cytokines had less or no effect.[7,17] Inflammatory cells in atherosclerotic plaques produce abundant tissue factor and upon plaque rupture there is extensive tissue factor exposure to blood.[18] In severe sepsis, mononuclear cells, stimulated by pro-inflammatory cytokines, express tissue factor, which leads to systemic activation of coagulation.[19] Even in experimental low-dose endotoxaemia in healthy subjects, a 125-fold increase in tissue factor mRNA levels in blood monocytes can be detected.[20] A potential alternative source of tissue factor may be endothelial cells, polymorphonuclear cells, and other cell types. It is hypothesised that tissue factor from these sources is shuttled between cells through microparticles derived from activated mononuclear cells.[21] It is, however, unlikely that these cells actually synthesise tissue factor in substantial quantities.[19,22]

Upon exposure to blood, tissue factor binds to factor VIIa. The complex of tissue factor-factor VIIa catalyses the conversion of factor X to Xa, which will form the prothrombinase complex with factor Va, prothrombin (factor II) and calcium, thereby generating thrombin (factor IIa). One of the key functions of thrombin is to convert fibrinogen into fibrin. The tissue factor-factor VIIa complex can also activate factor IX, forming a tenase complex with activated factor IX and factor X, generating additional factor Xa, thereby forming an essential amplification loop. The assembly of the prothrombinase and tenase complex is markedly facilitated if a suitable phospholipid surface is available, ideally presented by activated platelets. In the setting of inflammation-induced activation of coagulation, platelets can be activated directly by endotoxin or by pro-inflammatory mediators, such as platelet activating factor. Thrombin itself is one of the strongest platelet activators *in vivo*.

Activation of platelets may also accelerate fibrin formation by another mechanism.[23] The expression of tissue factor

on monocytes is markedly stimulated by the presence of platelets and granulocytes in a P-selectin dependent reaction.[24] This effect may be the result of nuclear factor kappa B (NF-κB) activation induced by binding of activated platelets to neutrophils and mononuclear cells.[25] This cellular interaction also markedly enhances the production of IL-1b, IL-8, macrophage chemoattractant protein (MCP)-1, and tumour necrosis factor (TNF)-α.[26] The expression of P-selectin on the activated platelet membrane will mediate the adherence of platelets to endothelial cells and leucocytes.

IMPAIRED REGULATORY PATHWAYS IN INFECTION AND INFLAMMATION

Procoagulant activity is regulated by three important anticoagulant pathways: antithrombin (AT), the protein C system and tissue factor pathway inhibitor (TFPI). During inflammation-induced activation of coagulation, the function of all three pathways can be impaired.[27] The serine protease inhibitor antithrombin is the main inhibitor of thrombin and factor Xa. Without heparin, AT neutralises coagulation enzymes in a slow, progressive manner.[28] Heparin induces conformational changes in AT that result in at least a 1000-fold enhancement of AT activity. Thus, the clinical efficacy of heparin is attributed to its interaction with AT. Endogenous glycosaminoglycans, such as heparan sulphates, on the vessel wall also promote AT-mediated inhibition of thrombin and other coagulation enzymes. During severe inflammatory responses, AT levels are markedly decreased owing to impaired synthesis (as a result of a negative acute phase response), degradation by elastase from activated neutrophils, and – quantitatively most importantly – consumption as a consequence of ongoing thrombin generation.[29] Pro-inflammatory cytokines can also cause reduced synthesis of glycosaminoglycans on the endothelial surface, which will also contribute to reduced AT function, since these glycosaminoglycans can act as physiological heparin-like cofactors of AT.[30] Activated protein C (APC) appears to play a central role in the pathogenesis of sepsis and associated organ dysfunction.[31] There is ample evidence that an insufficient functioning of the protein C pathway contributes to the derangement of coagulation in sepsis.[32,33] The circulating zymogen protein C is activated by the endothelial cell-bound thrombomodulin once this is activated by thrombin.[34] APC acts in concert with its co-factor protein S to proteolytically degrade the essential coagulation co-factors Va and VIIIa, and in that manner functions as an effective anticoagulant. The endothelial protein C receptor (EPCR) not only accelerates the activation of protein C several-fold, but also serves as a receptor for APC, and binding of APC to this receptor may amplify its anticoagulant and anti-inflammatory effects.[35] A recent study has demonstrated that exposure of cultured endothelial cells to APC results in the release of microparticles that contain EPCR.[36] but the relevance of that observation for coagulation or inflammation is not yet clear. In patients with severe inflammation, the protein C system is malfunctioning at virtually all levels. First, plasma levels of the zymogen protein C are low or very low, due to impaired synthesis, consumption, and degradation by proteolytic enzymes, such as neutrophil elastase.[37-39] Furthermore, a significant down-regulation of thrombomodulin, caused by pro-inflammatory cytokines such as TNF-α and IL-1, has been demonstrated, resulting in diminished protein C activation.[40,41] Low levels of free protein S may further compromise an adequate function of the protein C system. In plasma, 60% of the co-factor protein S is complexed to a complement regulatory protein, C4b binding protein (C4bBP). Increased plasma levels of C4bBP as a consequence of the acute phase reaction in inflammatory diseases may result in a relative protein S deficiency, which further contributes to a procoagulant state during sepsis. Although it has been shown that the β-chain of C4bBP (which mainly governs the binding to protein S) is largely unaffected during the acute phase response,[42] support for this hypothesis comes from studies showing that the infusion of C4bBP in combination with a sublethal dose of *Escherichia coli* (*E. coli*) into baboons resulted in a lethal response with severe organ damage due to disseminated intravascular coagulation (DIC).[43] Finally, but importantly, in sepsis the EPCR has shown to be down-regulated, which may further negatively affect the function of the protein C system. Apart from these effects, sepsis may cause a resistance toward APC by other mechanisms, which are partly dependent on a sharp increase in factor VIII levels (released from endothelial cells), but partly occur by yet unidentified mechanisms.[45] In experimental models of severe infection fibrinolysis is activated, demonstrated by an initial activation of plasminogen activation, followed by a marked impairment caused by the release in blood of PAI-1.[16,46,47] The latter inhibitor strongly inhibits fibrinolysis causing a net procoagulant situation. The molecular basis is cytokine-mediated activation of vascular endothelial cells; TNFα and IL-1 decreased free tissue plasminogen activator (tPA) and increased PAI-1 production, TNFa increased total urokinase type plasminogen activator (uPA) production in endothelial cells.[48,49] Endotoxin and TNFα stimulated PAI-1 production in liver, kidney, lung and adrenals of mice. The net procoagulant state is illustrated by a late rise in fibrin breakdown fragments after *E. coli* challenge of baboons. Experimental data also indicate that the fibrinolytic mechanism is active in clearing fibrin from organs and circulation. Endotoxin-induced fibrin

formation in kidneys and adrenals was most dependent on a decrease in uPA.[50] PAI-1 knockout mice challenged with endotoxin did not develop thrombi in the kidney in contrast to wildtype animals.[49] Endotoxin administration to mice with a functionally inactive thrombomodulin gene (TMProArg mutation) and defective protein C activator cofactor function caused fibrin plugs in the pulmonary circulation, while wildtype animals did not develop macroscopic fibrin.[51] This phenomenon proved to be temporary, with detectable thrombi at four hours after endotoxin, and disappearance of clots at 24 hours in animals sacrificed at that time point. These experiments demonstrate that fibrinolytic action is required to reduce the extent of intravascular fibrin formation.

Fibrinolytic activity is markedly regulated by PAI-1, the principal inhibitor of this system. Recent studies have shown that a functional mutation in the PAI-1 gene, the 4G/5G polymorphism, not only influenced the plasma levels of PAI-1, but was also linked to clinical outcome of meningococcal septicaemia. Patients with the 4G/4G genotype had significantly higher PAI-1 concentrations in plasma and an increased risk of death.[52] Further investigations demonstrated that the PAI-1 polymorphism did not influence the risk of contracting meningitis as such, but probably increased the likelihood of developing septic shock from meningococcal infection.[53] These studies are the first evidence that genetically determined differences in the level of fibrinolysis influences the risk of developing complications of a Gram-negative infection. In other clinical studies in cohorts of patients with DIC, high plasma levels of PAI-1 were one of the best predictors of mortality.[54,55] These data suggest that activation of coagulation contributes to mortality in this situation, but as indicated earlier, the fact that PAI-1 is an acute phase protein, a higher plasma concentration may also be a marker of disease rather than a causal factor. Interestingly, platelet α-granules contain large quantities of PAI-1 and release PAI-1 upon their activation. Since platelets become activated in case of severe inflammation and infection, this may further increase the levels of PAI-1 and contribute to the fibrinolytic shut-off.

INFECTION-INDUCED THROMBOSIS AND VASCULAR COMPLICATIONS

Apart from the generalised response upon systemic inflammation as discussed above, specific infections may result in thrombohaemorrhagic syndromes, haemolytic uraemic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) or vasculitis.[56,57] Symptoms and signs may be dominated by bleeding, thrombosis, or both.[1,58,59] Clinically overt infection-induced activation of coagulation may occur in 30 to 50% of patients with Gram-negative sepsis.[60] Contrary to widely held belief, this may appear as common in patients with Gram-positive sepsis as in those with Gram-negative sepsis.[60,61] Activation of the coagulation system has also been documented for non-bacterial pathogens, i.e. viruses,[62,63] protozoa (malaria),[64,65] fungi[66] and spirochetes.[67]

Viral and bacterial infections may result in an enhanced risk for local thromboembolic disease, i.e. deep venous thrombosis or pulmonary embolism. In a thromboembolic prevention study of low-dose subcutaneous standard heparin for hospitalised patients with infectious diseases, morbidity due to thromboembolic disease was significantly reduced in the heparin group compared with the group receiving no prophylaxis. There was, however, no beneficial effect of prophylaxis on mortality due to thromboembolic complications.[68] In chronic viral diseases, such as cytomegalovirus (CMV) or human immuno-deficiency virus (HIV) infection, the risk of thromboembolic complications is relatively low.[69-71] Common infections, such as influenza and other forms of upper respiratory tract infections, have been shown to not only increase systemic levels of haemostatic proteins, but also to affect the incidence of pulmonary embolism, albeit to a modest extent.[72,73] Also, these conditions seem to predispose for the occurrence of ischaemic stroke.[74] A recent paper points to the fact that the enhanced thrombotic risk may be related to inflammation, either occurring on itself (e.g. as a consequence of an autoimmune disorder) or related to infection.[75]

Viral haemorrhagic fever is complicated by DIC in the most severe cases.[76-78] DIC is not frequently encountered in other viral infections but has been reported in cases of infection with rotavirus,[79,80] varicella, rubella, rubeola and influenza.[81-84] TTP and HUS, triggered by a viral or bacterial infection,[56,85] frequently lead to bleeding symptoms, but also platelet and fibrin thrombi may be generated in various organs, leading to prominent symptoms with organ dysfunction. In specific infections, such as viral haemorrhagic fever, bleeding complications are prominent.[76,77] In other viral and bacterial infections associated with TTP or HUS, bleeding is also often the prominent and presenting symptom.[55] Bacterial and viral infections may result in a vasculitis-like syndrome with either bleeding manifestations or ischaemic injury.[86-88] Vasculitis is a well-documented phenomenon in CMV infection,[89,90] occurring predominantly in the vasculature of the gastrointestinal tract where it causes colitis,[91,92] the central nervous system where it causes cerebral infarction,[93,94] and the skin where it results in petechiae, purpura papules, localised ulcers or a diffuse maculopapular eruption.[95] HIV infection may be accompanied by vasculitis syndromes, e.g. polyarteriitis nodosa, Henoch-Schönlein purpura and leucocytoclastic vasculitis.[96-98] Hepatitis B and C infection may cause

polyarteritis-like vasculitis.[99,100] Parvovirus B19 has been suggested to be associated with vasculitis-like syndromes including Kawasaki disease, polyarteritis nodosa and Wegener's granulomatosis.[101-103]

THERAPEUTIC IMPLICATIONS

Anticoagulant therapy in patients with severe infection remains controversial. Experimental studies have shown that heparin can at least partly inhibit the activation of coagulation in severe sepsis and other infections. However, a beneficial effect of heparin on clinically important outcome events in patients with DIC has not been demonstrated in controlled clinical trials. Also, the safety of heparin treatment is debatable in patients with haemorrhagic complications of infection, such as in some viral diseases or in DIC, who are prone to bleeding.[104] A large trial in patients with severe sepsis showed a slight but non-significant benefit of low-dose heparin on 28-day mortality in patients with severe sepsis and no major safety concerns.[105] There is general consensus that administration of heparin is beneficial in some categories of infection-related procoagulant states. Heparin is obviously indicated for treating thromboembolic complications in large vessels in patients with inflammation and infection. Heparin administration may be helpful in patients with acute DIC when intensive blood component replacement fails to improve excessive bleeding or when thrombosis threatens to cause irreversible tissue injury (e.g., acute cortical necrosis of the kidney or digital gangrene).

Theoretically, the most logical anticoagulant agent to use in the setting of hypercoagulability in the setting of infection or inflammation is directed against tissue factor activity. Potential agents include recombinant TFPI, inactivated factor VIIa, and recombinant nematode anticoagulant protein c2 (NAPc2), a potent and specific inhibitor of the ternary complex of TF/factor VIIa and factor Xa. Phase II trials of recombinant TFPI in patients with sepsis showed promising results but phase III trials in patients with severe sepsis or severe pneumonia and organ failure did not show an overall survival benefit in patients who were treated with TFPI.[106] Recombinant human soluble thrombomodulin binds to thrombin to form a complex that inactivates thrombin's coagulant activity and activates protein C and, thus, is a potential drug for the treatment of patients with DIC. In a phase III randomised double-blind clinical trial in patients with DIC, administration of the soluble thrombomodulin had a significantly better effect on bleeding manifestations and coagulation parameters than heparin. Currently ongoing trials with soluble thrombomodulin focus on DIC, organ failure and mortality rate.

CONCLUSION

There is a tight link between infection and inflammation on the one hand and activation of coagulation and venous and arterial thrombosis on the other hand. Pro-inflammatory cytokines are crucial in mediating these effects. The interaction between inflammation and coagulation involves significant cross-talk between the systems and seems to occur at the interface formed by endothelial cells. Several mechanisms contribute to an enhanced risk of both venous thromboembolism and accelerated atherosclerosis in patients with infections and (chronic) inflammation. Although it is likely that anticoagulant treatment is important to prevent infection- and inflammation-associated thrombotic complications, clinical evidence of efficacy and safety of this approach is still limited.

REFERENCES

1 Levi M, ten Cate H. Disseminated intravascular coagulation. N Engl J Med. 1999;341:586-92.

2 Wheeler AP, Bernard GR. Treating patients with severe sepsis. N Engl J Med. 1999;340:207-14.

3 Levi M, Keller TT, van Gorp E, ten Cate H. Infection and inflammation and the coagulation system. Cardiovasc Res. 2003;60:26-39.

4 Levi M, Schultsz M, van der Poll T. Infection and inflammation as a risk factor for thrombosis. Sem Thromb Hemostas. 2012; in press.

5 Levi M, van der Poll T. Inflammation and coagulation. Crit Care Med. 2010;38:S26-S34.

6 Esmon CT, Fukudome K, Mather T, et al. Inflammation, sepsis, and coagulation. Haematologica. 1999;84:254-9.

7 Levi M, van der Poll T, ten Cate H, van Deventer SJ. The cytokine-mediated imbalance between coagulant and anticoagulant mechanisms in sepsis and endotoxaemia. Eur J Clin Invest. 1997;27:3-9.

8 Osterud B, Bjorklid E. The tissue factor pathway in disseminated intravascular coagulation. Semin Thromb Hemost. 2001;27:605-17.

9 Taylor FB. Response of anticoagulant pathways in disseminated intravascular coagulation. Semin Thromb Hemost. 2001;27:619631.

10 ten Cate JW, van der Poll T, Levi M, et al. Cytokines: triggers of clinical thrombotic disease. Thromb Haemost. 1997;78:415-9.

11 Aird WC. Vascular bed-specific hemostasis: role of endothelium in sepsis pathogenesis. Crit Care Med. 2001;29:S28-S34.

12 Levi M, ten Cate H, van der Poll T. Endothelium: interface between coagulation and inflammation. Crit Care Med. 2002;30:S220-S224.

13 Vallet B. Microthrombosis in sepsis. Minerva Anestesiol. 2001;67:298-301.

14 Levi M, van der Poll T, ten Cate H. Tissue factor in infection and severe inflammation. Semin Thromb Hemost. 2006;32:33-9.

15 Taylor FBJ, Chang A, Ruf W, et al. Lethal E. coli septic shock is prevented by blocking tissue factor with monoclonal antibody. Circ Shock. 1991;33:127-34.

16 Levi M, ten Cate H, Bauer KA, et al. Inhibition of endotoxin-induced activation of coagulation and fibrinolysis by pentoxifylline or by a monoclonal anti-tissue factor antibody in chimpanzees. J Clin Invest. 1994;93:114-20.

17 van der Poll T, Levi M, Hack CE, et al. Elimination of interleukin 6 attenuates coagulation activation in experimental endotoxemia in chimpanzees. J Exp Med. 1994;179:1253-9.

18 Libby P, Aikawa M. Stabilization of atherosclerotic plaques: new mechanisms and clinical targets. Nat Med. 2002;8:1257-62.

19 Osterud B, Rao LV, Olsen JO. Induction of tissue factor expression in whole blood – lack of evidence for the presence of tissue factor expression on granulocytes. Thromb Haemost. 2000;83:861-7.

20 Franco RF, de Jonge E, Dekkers PE, et al. The in vivo kinetics of tissue factor messenger RNA expression during human endotoxemia: relationship with activation of coagulation. Blood. 2000;96:554-9.

21 Rauch U, Bonderman D, Bohrmann B, et al. Transfer of tissue factor from leukocytes to platelets is mediated by CD15 and tissue factor. Blood. 2000;96:170-5.

22 Osterud B, Bjorklid E. Sources of tissue factor. Semin Thromb Hemost. 2006;32:11-23.

23 Lowenberg EC, Meijers JC, Levi M. Platelet-vessel wall interaction in health and disease. Neth J Med. 2010;68:242-51.

24 Osterud B. Tissue factor expression by monocytes: regulation and pathophysiological roles. Blood Coagul Fibrinolysis. 1998;9 Suppl 1:S9-14.

25 Furie B, Furie BC. Role of platelet P-selectin and microparticle PSGL-1 in thrombus formation. Trends Mol Med. 2004;10:171-8.

26 Neumann FJ, Marx N, Gawaz M, et al. Induction of cytokine expression in leukocytes by binding of thrombin-stimulated platelets. Circulation. 1997;95:2387-94.

27 Levi M, van der Poll T. The role of natural anticoagulants in the pathogenesis and management of systemic activation of coagulation and inflammation in critically ill patients. Semin Thromb Hemost. 2008;34:459-68.

28 Levi M. Antithrombin in sepsis revisited. Crit Care. 2005;9:624-5.

29 Levi M, van der Poll T, Buller HR. Bidirectional relation between inflammation and coagulation. Circulation. 2004;109:2698-704.

30 Kobayashi M, Shimada K, Ozawa T. Human recombinant interleukin-1 beta- and tumor necrosis factor alpha-mediated suppression of heparin-like compounds on cultured porcine aortic endothelial cells. J Cell Physiol. 1990;144:383-90.

31 Levi M, van der Poll T. Recombinant human activated protein C: current insights into its mechanism of action. Crit Care. 2007;11 Suppl 5:S3:S3.

32 Esmon CT. Role of coagulation inhibitors in inflammation. Thromb Haemost. 2001;86:51-6.

33 Levi M, de Jonge E, van der Poll T. Rationale for restoration of physiological anticoagulant pathways in patients with sepsis and disseminated intravascular coagulation. Crit Care Med. 2001;29:S90-S94.

34 Esmon CT. The regulation of natural anticoagulant pathways. Science. 1987;235:1348-52.

35 Esmon CT. The endothelial cell protein C receptor [Review.] Thromb Haemost. 2000;83:639-43.

36 Perez-Casal M, Downey C, Fukudome K, Marx G, Toh CH. Activated protein C induces the release of microparticle-associated endothelial protein C receptor. Blood. 2005;105:1515-22.

37 Mesters RM, Helterbrand J, Utterback BG, et al. Prognostic value of protein C concentrations in neutropenic patients at high risk of severe septic complications. Crit Care Med. 2000;28:2209-16.

38 Vary TC, Kimball SR. Regulation of hepatic protein synthesis in chronic inflammation and sepsis. Am J Physiol. 1992;262:C445-C452.

39 Eckle I, Seitz R, Egbring R, Kolb G, Havemann K. Protein C degradation in vitro by neutrophil elastase. Biol Chem Hoppe Seyler. 1991;372:1007-13.

40 Nawroth PP, Stern DM. Modulation of endothelial cell hemostatic properties by tumor necrosis factor. J Exp Med. 1986;163:740-5.

41 Faust SN, Levin M, Harrison OB, et al. Dysfunction of endothelial protein C activation in severe meningococcal sepsis. N Engl J Med. 2001;345:408-16.

42 Garcia de Frutos P, Alim RI, Hardig Y, et al. Differential regulation of alpha and beta chains of C4b-binding protein during acute-phase response resulting in stable plasma levels of free anticoagulant protein S. Blood. 1994;84:815-22.

43 Taylor FBJ, Dahlback B, Chang AC, et al. Role of free protein S and C4b binding protein in regulating the coagulant response to Escherichia coli. Blood. 1995;86:2642-52.

44 Taylor FBJ, Stearns-Kurosawa DJ, Kurosawa S, et al. The endothelial cell protein C receptor aids in host defense against Escherichia coli sepsis. Blood. 2000;95:1680-6.

45 De Pont AC, Bakhtiari K, Hutten BA, et al. Endotoxaemia induces resistance to activated protein C in healthy humans. Br J Haematol. 2006;134:213-9.

46 Biemond BJ, Levi M, ten Cate H, et al. Plasminogen activator and plasminogen activator inhibitor I release during experimental endotoxaemia in chimpanzees: effect of interventions in the cytokine and coagulation cascades. Clin Sci (Colch). 1995;88:587-94.

47 Biemond BJ, Friederich PW, Koschinsky ML, et al. Apolipoprotein(a) attenuates endogenous fibrinolysis in the rabbit jugular vein thrombosis model in vivo. Circulation. 1997;96:1612-5.

48 Schleef RR, Bevilacqua MP, Sawdey M, et al. Cytokine activation of vascular endothelium. Effects on tissue-type plasminogen activator and type 1 plasminogen activator inhibitor. J Biol Chem. 1988;263:5797-803.

49 Sawdey MS, Loskutoff DJ. Regulation of murine type 1 plasminogen activator inhibitor gene expression in vivo. Tissue specificity and induction by lipopolysaccharide, tumor necrosis factor-alpha, and transforming growth factor-beta. J Clin Invest. 1991;88:1346-53.

50 Yamamoto K, Loskutoff DJ. Fibrin deposition in tissues from endotoxin-treated mice correlates with decreases in the expression of urokinase-type but not tissue-type plasminogen activator. J Clin Invest. 1996;97:2440-51.

51 ten Cate H. Pathophysiology of disseminated intravascular coagulation in sepsis. Crit Care Med. 2000;S9-S11.

52 Hermans PW, Hibberd ML, Booy R, et al. 4G/5G promoter polymorphism in the plasminogen-activator-inhibitor-1 gene and outcome of meningococcal disease. Meningococcal Research Group. Lancet. 1999;354:556-60.

53 Westendorp RG, Hottenga JJ, Slagboom PE. Variation in plasminogen-activator-inhibitor-1 gene and risk of meningococcal septic shock. Lancet. 1999;354:561-3.

54 Brandtzaeg P, Joo GB, Brusletto B, Kierulf P. Plasminogen activator inhibitor 1 and 2, alpha-2-antiplasmin, plasminogen, and endotoxin levels in systemic meningococcal disease. Thromb Res. 1990;57:271-8.

55 Mesters RM, Florke N, Ostermann H, Kienast J. Increase of plasminogen activator inhibitor levels predicts outcome of leukocytopenic patients with sepsis. Thromb Haemost. 1996;75:902-7.

56 Zimmerhackl LB, Rosales A, Hofer J, et al. Enterohemorrhagic Escherichia coli O26:H11-Associated Hemolytic Uremic Syndrome: Bacteriology and Clinical Presentation. Semin Thromb Hemost. 2010;36:586-93.

57 Karpman D, Sartz L, Johnson S. Pathophysiology of typical hemolytic uremic syndrome. Semin Thromb Hemost. 2010;36:575-85.

58 van Gorp E, Suharti C, ten Cate H, et al. Review: infectious diseases and coagulation disorders. J Infect Dis. 1999;180:176-86.

59 Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. Nature. 2000;406:782-87.

60 Anas AA, Wiersinga WJ, de Vos AF, van der Poll T. Recent insights into the pathogenesis of bacterial sepsis. Neth J Med. 2010;68:147-52.

61 Vos FJ, Bleeker-Rovers CP, Sturm PD, et al. Endocarditis: effects of routine echocardiography during Gram-positive bacteraemia. Neth J Med. 2011;69:335-40.

62 Bhamarapravati N. Hemostatic defects in dengue hemorrhagic fever. Rev Infect Dis. 1989;11 Suppl 4:S826-S829.

63 Heller MV, Marta RF, Sturk A, et al. Early markers of blood coagulation and fibrinolysis activation in Argentine hemorrhagic fever. Thromb Haemost. 1995;73:368-73.

64 Mohanty D, Ghosh K, Nandwani SK, et al. Fibrinolysis, inhibitors of blood coagulation, and monocyte derived coagulant activity in acute malaria. Am J Hematol. 1997;54:23-9.

65 Chuttani K, Tischler MD, Pandian NG, et al. Diagnosis of cardiac tamponade after cardiac surgery: relative value of clinical, echocardiographic, and hemodynamic signs. Am Heart J. 1994;127:913-8.

66 Fera G, Semeraro N, De Mitrio V, Schiraldi O. Disseminated intravascular coagulation associated with disseminated cryptococcosis in a patient with acquired immunodeficiency syndrome. Infection. 1993;21:171-3.

67 Schroder S, Spyridopoulos I, Konig J, et al. Thrombotic thrombocytopenic purpura (TTP) associated with a Borrelia burgdorferi infection. Am J Hematol. 1995;50:72-3.

68 Gardlund B. Randomised, controlled trial of low-dose heparin for prevention of fatal pulmonary embolism in patients with infectious diseases. The Heparin Prophylaxis Study Group.[see comments] Lancet. 1996;347:1357-61.

69 Laing RB, Brettle RP, Leen CL. Venous thrombosis in HIV infection. [editorial] Int J STD AIDS. 1996;7:82-5.

70 Jenkins RE, Peters BS, Pinching AJ. Thromboembolic disease in AIDS is associated with cytomegalovirus disease.[letter] AIDS. 1991;5:1540-2.

71 Drancourt M, George F, Brouqui P, Sampol J, Raoult D. Diagnosis of Mediterranean spotted fever by indirect immunofluorescence of Rickettsia conorii in circulating endothelial cells isolated with monoclonal antibody-coated immunomagnetic beads. J Infect Dis. 1992;166:660-3.

72 Keller TT, van Wissen M, Mairuhu AT, van Doornum GJ, Brandjes DP. Acute respiratory tract infections in elderly patients increase systemic levels of hemostatic proteins. J Thromb Haemost. 2007;5:1567-9.

73 van Wissen M, Keller TT, Ronkes B, et al. Influenza infection and risk of acute pulmonary embolism. Thromb J. 2007;5:16.

74 Grau AJ, Urbanek C, Palm F. Common infections and the risk of stroke. Nat Rev Neurol. 2010;6:681-94.

75 Zoller B, Li X, Sundquist J, Sundquist K. Risk of pulmonary embolism in patients with autoimmune disorders: a nationwide follow-up study from Sweden. Lancet. 2011.

76 Hayes EB, Gubler DJ. Dengue and dengue hemorrhagic fever.[Review] Pediatr Infect Dis J. 1992;11:311-7.

77 Sumarmo, Wulur H, Jahja E et al. Clinical observations on virologically confirmed fatal dengue infections in Jakarta, Indonesia. Bull World Health Organ. 1983;61:693-701.

78 Kuberski T, Rosen L, Reed D, Mataika J. Clinical and laboratory observations on patients with primary and secondary dengue type 1 infections with hemorrhagic manifestations in Fiji. Am J Trop Med Hyg. 1977;26:775-83.

79 Limbos MA, Lieberman JM. Disseminated intravascular coagulation associated with rotavirus gastroenteritis: report of two cases.[see comments] Clin Infect Dis. 1996;22:834-36.

80 Anderson DR, Harrison L, Hirsh J. Evaluation of a portable prothrombin time monitor for home use by patients who require long-term oral anticoagulant therapy. Arch Intern Med. 1993;153:1441-7.

81 McKay DG, Margaretten W. Disseminated intravascular coagulation in virus diseases. Arch Intern Med. 1967;120:129-52.

82 Linder M, Muller-Berghaus G, Lasch HG, Gagel C. Virus infection and blood coagulation. Thromb Diath Haemorrh. 1970;23:1-11.

83 Anderson DR, Schwartz J, Hunter NJ, et al. Varicella hepatitis: a fatal case in a previously healthy, immunocompetent adult. Report of a case, autopsy, and review of the literature. Arch Intern Med. 1994;154:2101-6.

84 Clemens R, Pramoolsinsap C, Lorenz R, et al. Activation of the coagulation cascade in severe falciparum malaria through the intrinsic pathway. Br J Haematol. 1994;87:100-5.

85 Badesha PS, Saklayen MG. Hemolytic uremic syndrome as a presenting form of HIV infection. Nephron. 1996;72:472-5.

86 Ackerman AB, Chongchitnant N, Sanchez J, Guo Y. Inflammatory diseases; Histologic diagnosis of inflammatory skin diseases. Williams and Wilkins. 1997;170-86.

87 Lie JT. Vasculitis associated with infectious agents. Curr Opin Rheumatol. 1996;8:26-9.

88 Guillevin L, Lhote F, Gherardi R. The spectrum and treatment of virus-associated vasculitides. Curr Opin Rheumatol. 1997;9:31-6.

89 Golden MP, Hammer SM, Wanke CA, Albrecht MA. Cytomegalovirus vasculitis. Case reports and review of the literature. Medicine. 1994;73:246-55.

90 Ho DD, Rota TR, Andrews CA, Hirsch MS. Replication of human cytomegalovirus in endothelial cells. J Infect Dis. 1984;150:956-7.

91 Goodman MD, Porter DD. Cytomegalovirus vasculitis with fatal colonic hemorrhage. Arch Pathol Lab Med. 1973;96:281-4.

92 Foucar E, Mukai K, Foucar K, et al. Colon ulceration in lethal cytomegalovirus infection. Am J Clin Pathol. 1981;76:788-801.

93 Booss J, Dann PR, Winkler SR, et al. Mechanisms of injury to the central nervous system following experimental cytomegalovirus infection. Am J Otolaryngol. 1990;11:313-7.

94 Koeppen AH, Lansing LS, Peng SK, Smith RS. Central nervous system vasculitis in cytomegalovirus infection. J Neurol Sci. 1981;51:395-410.

95 Lin CS, Penha PD, Krishnan MN, Zak FG. Cytomegalic inclusion disease of the skin. Arch Dermatol. 1981;117:282-4.

96 Libman BS, Quismorio FPJ, Stimmler MM. Polyarteritis nodosa-like vasculitis in human immunodeficiency virus infection. J Rheumatol. 1995;22:351-5.

97 Calabrese LH. Vasculitis and infection with the human immunodeficiency virus. Rheum Dis Clin North Am. 1991;17:131-47.

98 Gherardi R, Belec L, Mhiri C, et al. The spectrum of vasculitis in human immunodeficiency virus-infected patients. A clinicopathologic evaluation. Arthritis Rheum. 1993;36:1164-74.

99 Sergent JS, Lockshin MD, Christian CL, et al. Vasculitis with hepatitis B antigenemia: long-term observation in nine patients. Medicine. 1976;55:1-18.

100 Carson CW, Conn DL, Czaja AJ, et al. Frequency and significance of antibodies to hepatitis C virus in polyarteritis nodosa. J Rheumatol. 1993;20:304-9.

101 Leruez-Ville M, Lauge A, Morinet F, et al. Polyarteritis nodosa and parvovirus B19. Lancet. 1994;344:263-4.

102 Nikkari S, Mertsola J, Korvenranta H, et al. Wegener's granulomatosis and parvovirus B19 infection. Arthritis Rheum. 1994;37:1707-8.

103 Yoto Y, Kudoh T, Haseyama K, et al. Human parvovirus B19 infection in Kawasaki disease.[letter; comment] Lancet. 1994;344:58-9.

104 Levi MM, Eerenberg E, Lowenberg E, Kamphuisen PW. Bleeding in patients using new anticoagulants or antiplatelet agents: risk factors and management. Neth J Med. 2010;68:68-76.

105 Levi M, Levy M, Williams MD, et al. Prophylactic heparin in patients with severe sepsis treated with drotrecogin alfa (activated). Am J Respir Crit Care Med. 2007;176:483-90.

106 Abraham E, Reinhart K, Opal S, et al. Efficacy and safety of tifacogin (recombinant tissue factor pathway inhibitor) in severe sepsis: a randomized controlled trial. JAMA. 2003;290:238-47.

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REVIEW

A new era in the diagnosis and treatment of atypical haemolytic uraemic syndrome

D. Westra[1], J.F.M. Wetzels[2], E.B. Volokhina[1], L.P. van den Heuvel[1], N.C.A.J. van de Kar[1]*

Departments of [1]Paediatric Nephrology and [2]Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, *corresponding author: tel: +31 (0)24-3614430, fax: +31 (0)24-3616428, n.vandekar@cukz.umcn.nl

ABSTRACT

The haemolytic uraemic syndrome (HUS) is characterised by haemolytic anaemia, thrombocytopenia and acute renal failure. The majority of cases are seen in childhood and are preceded by an infection with Shiga-like toxin producing *Escherichia coli* (STEC-HUS; so-called typical HUS). Non-STEC or atypical HUS (aHUS) is seen in 5 to 10% of all cases and occurs at all ages. These patients have a poorer outcome and prognosis than patients with STEC-HUS. New insights into the pathogenesis of aHUS were revealed by the identification of mutations in genes encoding proteins of the alternative pathway of the complement system in aHUS patients. Specific information of the causative mutation is important for individualised patient care with respect to choice and efficacy of therapy, the outcome of renal transplantation, and the selection of living donors. This new knowledge about the aetiology of the disease has stimulated the development of more specific treatment modalities. Until now, plasma therapy was used with limited success in aHUS, but recent clinical trials have demonstrated that patients with aHUS can be effectively treated with complement inhibitors, such as the monoclonal anti-C5 inhibitor eculizumab.

KEYWORDS

Atypical haemolytic uraemic syndrome, complement system, eculizumab, kidney transplantation, plasma therapy

INTRODUCTION

The haemolytic uraemic syndrome (HUS) is a rare and severe thrombotic microangiopathy (TMA) characterised by the triad of haemolytic anaemia, thrombocytopenia and acute renal failure. HUS is characterised histologically by vascular abnormalities with glomerular endothelial damage, swelling of the endothelium, endothelial detachment of the basement membrane, intima fibrosis, and thrombosis. In *table 1,* the most important causes of thrombotic microangiopathies (both HUS and thrombotic thrombocytopenic purpura [TTP]) are shown.[1,2]

Table 1. *Causes of haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura*

Infectious
- Infection with Shiga-like toxin producing *Escherichia coli* (*STEC*)
- Infection with neuraminidase producing *Streptococcus pneumoniae*
- Human immunodeficiency virus (HIV)

Complement dysregulation
- Genetic abnormalities in complement (regulating) proteins
- Acquired defects (autoantibodies against CFH)

ADAMTS13 deficiency
- Genetic abnormalities
- Autoantibodies against ADAMTS13

Clinically associated with
- *Systemic diseases*: SLE, antiphospholipid syndrome, defective cobalamin metabolism
- *Medication*: ticlopedin, mitomycin, bleomycin, cysplatin, quinine, tacrolimus, cyclosporin, rifampicin, clopidopogrel
- *Malignancies*: chemotherapy
- *Viruses*: cytomegalovirus, parvovirus
- *Transplantation*: calcineurin inhibitors, rejection
- *Pregnancy*: oral contraceptives, pre-eclampsia, HELLP syndrome
- *Glomerulopathies*: MPGN type II
- *Bone marrow transplantation*: radiation, medication, graft vs host disease

ADAMTS13 = a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; CFH = complement factor H; HELLP = haemolysis, elevated liver enzymes and low platelets; MPGN = membranoproliferative glomerulonephritis; SLE = systemic lupus erythematosus.

In more than 90% of the cases, the disease is triggered by an infection with Shiga-like toxin producing *Escherichia coli* (STEC). Especially young children between two and six years of age are sensitive to the development of the disease. Three to eight days after contamination with the bacteria, the patient develops abdominal pain with watery and/or bloody diarrhoea, followed within 24 hours by haemolytic anaemia, thrombocytopenia, and acute renal failure. This HUS is also called typical or diarrhoea-associated HUS (D^+ HUS) or STEC-HUS. Mortality in children with STEC-HUS is 3 to 5% during the acute phase of the disease;[3] about 75% of the patients completely recover after an episode of STEC-HUS.[4]

While STEC-HUS is indeed mostly seen in children, in the recent world's largest STEC outbreak in Germany, mostly adults above 20 years and predominantly females were affected. This was attributed to the changes in the microbial characteristics of the bacteria (STEC O104:H4), which shares virulence characteristics of typical STEC strains and enteroaggregative *E. coli* strains,[5] indicating that changes in the bacterial characteristics can lead to changes in host profile. Although a greater proportion of patients infected with STEC O104:H4 eventually developed HUS,[5] both clinical course of individual patients and mortality (~4%) seemed to be comparable with historic reports.[5,6]

Non-STEC-HUS is seen in 5 to 10% of all HUS cases, can appear at any age and may be sporadic or familial. These patients have a poor prognosis with a high mortality and morbidity in the acute phase of the disease and progression to end-stage renal disease (ESRD) in 50% of the cases.[7,8] Many causes of this so-called atypical HUS (aHUS) have been identified (*table 1*). The recently recognised disorders of complement regulation will be outlined in this review. Other associations include various non-enteric infections (especially *Streptococcus pneumoniae* infections), viruses, malignancies, drugs, bone marrow and kidney transplantation, pregnancy, and systemic diseases.

Atypical HUS needs to be distinguished from TTP, although they overlap clinically and morphologically. Both diseases share features of a thrombotic microangiopathy, caused by activation and damage of endothelial cells. In aHUS this is mostly confined to the glomerular endothelium, while in TTP there is more systemic vascular endothelial damage. Histochemical studies revealed that thrombi of patients with TTP mostly contain thrombocytes, while HUS thrombi are positive for fibrin instead of platelets.[9] Neurological symptoms and thrombocytopenia prevail in TTP, while kidney failure is limited. In aHUS, on the other hand, kidney failure is the most important clinical symptom. On clinical grounds it may be difficult to differentiate between aHUS and TTP and this may cause a delay in treatment. The discovery of the specific involvement of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) in the pathogenesis of TTP has allowed discrimination between the two TMAs: in patients with TTP, ADAMTS13 activity is greatly reduced (5 to 10% of normal). This is mostly due to autoantibodies against ADAMTS13. Congenital TTP, caused by mutations in the ADAMTS13 gene, is an extremely rare autosomal recessive disease (incidence 1:1,000,000), which manifests often, but not exclusively, at birth or during childhood.[10] Ten to 25% of TTP patients, however, have normal ADAMTS13 activity, suggesting the presence of as yet unknown physiopathological mechanisms.

In this review, we will further focus on the role of the complement system in the pathogenesis, outcome, and treatment of atypical HUS.

THE COMPLEMENT SYSTEM AND ATYPICAL HUS

Already in the 1970s decreased plasma levels of the complement proteins C3 and complement factor B (CFB) in both sporadic and familial cases of HUS were identified.[11] The presence of increased breakdown products of these proteins suggested that activation of the alternative pathway of the complement system could be involved in the pathogenesis of the disease.[12] In the last decade, indeed, a clear link was demonstrated between aHUS and genetic abnormalities in complement (regulating) genes, which can result in hyperactivation of the complement system, eventually leading to glomerular endothelial activation and thrombosis.

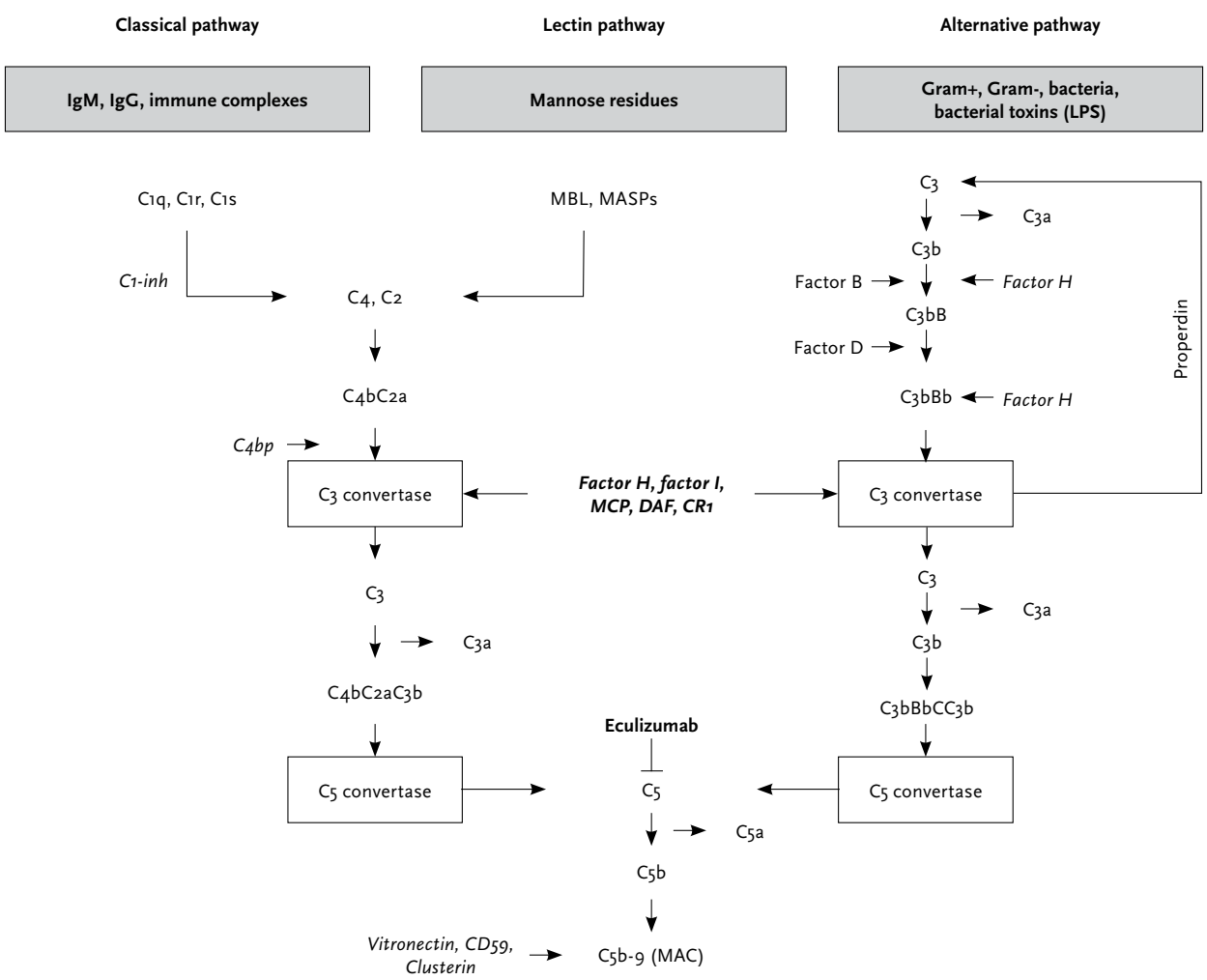
Activation and regulation of the complement system

The human complement system is part of the innate immunity and consists of more than 40 plasma and membrane-associated proteins. The most important roles of the complement system are the recognition of pathogens (opsonisation), the activation and chemotaxis of leucocytes, and the induction of cell lysis by incorporation of the membrane attack complex (MAC).[13,14] Three activation pathways are recognised: the classical pathway, the mannose binding lectin pathway, and the alternative pathway. In aHUS, the alternative pathway is mostly affected.

To prevent continued and unopposed complement activation, and resulting cell damage, the complement system is tightly regulated. Each pathway has its own regulators (inhibitors), but some regulators work on more than one pathway (*figure 1;* inhibitors shown in italic). Activating regulators include complement factor B and complement factor D.

Foreign surfaces that either lack membrane-bound regulators or cannot bind soluble regulators are attacked

Figure 1. *Schematic overview of the three activation pathways of the complement system*



Activation via the classical, lectin, or alternative pathway leads to production of C3 convertases, which can cleave C3 in C3b and the anaphylatoxin C3a. Active C3b finally initiates the production of membrane attack complexes that can cause lysis of the cells. The regulators of the complement system, important in the protection of host cells against complement activation, are shown in italic. C1-inh = C1 inhibitor; C4bp = C4 binding protein; CR1 = complement receptor-1; DAF = decay accelerating factor; MAC = membrane attack complex; MASP = mannose associated serine protease; MBL = mannose binding lectin; MCP = membrane cofactor protein.

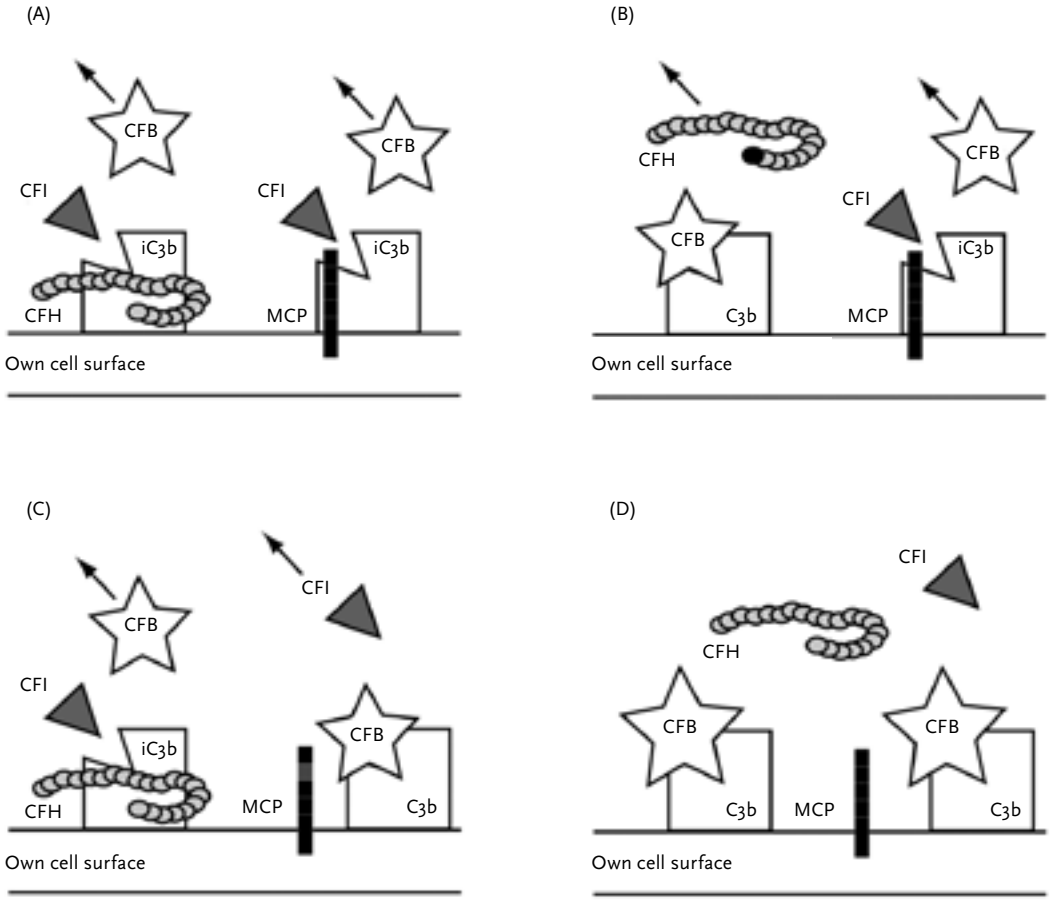
and damaged by the complement system. The key regulators of the alternative pathway are complement factor H (CFH), complement factor I (CFI), and membrane co-factor protein (MCP or CD46). These complement regulatory proteins are either constitutively present on the endothelial cell membrane or are bound by the endothelial glycocalyx. The mechanism of complement regulation at the cell surface by these regulators is schematically shown in *figure 2A*.

Mutations in complement genes in aHUS patients

A loss-of-function mutation in a complement-inhibiting gene or a gain-of-function mutation in a gene that encodes a complement activator will lead to an unopposed activation of the complement system, resulting in formation of the membrane attack complex on cell surfaces of especially endothelial cells in the microcirculation of the kidney. As a result, endothelial cells are damaged and leucocytes are attracted, releasing oxygen radicals and proteinases, which can further damage the endothelium. This will eventually result in increased platelet adherence and the formation of microthrombi in the kidney, thus explaining the characteristic triad of aHUS: acute renal failure, thrombocytopenia, and haemolytic anaemia.

In 1998, Warwicker *et al.* were the first to describe a mutation in the gene encoding CFH in familial cases of aHUS.[15] Nowadays, a genetic aberration in one of the proteins of the alternative complement pathway can be found in at least 50% of the aHUS patients. Mutations, usually heterozygous, have been identified

Figure 2. *Schematic model for the mechanism of alternative complement pathway regulation at host cell surfaces*



In normal circumstances small amounts of C3b are deposited on cell surfaces, but these molecules are rapidly eliminated by CFI, with the help of the cofactors CFH and MCP (A). In case of a loss-of-function mutation in CFH (B) or MCP (C), deposited C3b on host cells cannot be efficiently eliminated. This is followed by complement activation that can lead to complement-mediated damage of plasma exposed cells, such as glomerular endothelial cells. A gain-of-function mutation in a molecule that participates in activation of the alternative pathway, endothelial cells will be damaged despite the presence of functional regulators (D). Adapted by permission from Elsevier Limited.[8]

in the complement inhibitors CFH, CFI, MCP, and in only one single study in thrombomodulin (THBD), and in the activators C3 and CFB.[16-22] In addition, aHUS is associated with the presence of a combination of single nucleotide polymorphisms in CFH (CFH_{TGTGT} haplotype) or MCP (MCP_{GGAAC} haplotype).[23] Not unexpectedly, aHUS can also be caused by antibodies that impair the action of the complement regulatory proteins. Thus far, autoantibodies against CFH (αFH) have been identified in aHUS patients.[24] These αFH autoantibodies can block the epitopes of CFH that are involved in binding to the endothelial cell membrane, resulting in defective regulation of the complement at the site of the endothelium, leading to endothelial damage.[25] The development of these αFH antibodies is associated with a polymorphic homozygous deletion of complement factor H related proteins (CFHR1 and CFHR3).[24] Mechanisms of disease for several mutations are shown in *figures 2B-D*.

Complement investigations in aHUS patients

Recent guidelines suggest screening for complement abnormalities in patients with aHUS.[26,27] Complement activity (CH50 and AP50) and serum complement components (C3, C4, C3d, CFH, and CFI) can be measured in serum, drawn before the start of therapy. It must be realised that most assays measure the presence of the protein and not the activity. Moreover, abnormalities in complement regulation may only occur at the level of the endothelial cell surface, and not systemically. Therefore, serum levels of the above-mentioned complement components may be normal in patients with complement dysregulation and thus cannot exclude a genetic complement disorder.[7,18,28] The surface expression of membrane-bound MCP on mononuclear leucocytes can be investigated by fluorescent-activated cell sorting (FACS). Mutational screening should be performed in the complement genes that have been associated with aHUS

(*CFH*, *CFI*, *MCP*, *C3*, *CFB*, and *THBD*), irrespective of serum C3, CFH, or CFI levels. The presence of αFH can be identified in serum by enzyme-linked immunosorbent assay (ELISA). ADAMTS13 activity should be measured to exclude TTP: an activity below 5 to 10% could indicate acquired anti-ADAMTS13 autoantibodies (in the majority of the patients) or a genetic abnormality. The possibilities of a rare cause of aHUS, such as HIV infection, pregnancy, or cobalamin deficiency, should be considered and investigated at presentation. STEC infection has to be ruled out in aHUS patients as well, as an unusual presentation of STEC-HUS can occur. In 10% of the patients no diarrhoea occurs [29] and STEC-HUS can occur in adults as well, as in the German outbreak. An overview of the investigations to be performed in patients with aHUS is shown in *table 2*.

Table 2. *Overview of investigations to be performed in patients diagnosed with atypical HUS*

Underlying cause of TMA	Technique
Disorders of complement regulation	
C3 and C4 levels	Nephelometry (serum)*
C3d levels	Immuno-electrophoresis (EDTA plasma)*,†
CFH and CFI levels	Radial immunodiffusion (serum)*
Autoantibodies against CFH	ELISA (serum)*
Surface expression MCP	FACS (EDTA blood)
Mutational screening CFH, CFI, MCP, C3, and CFB	Sequencing analysis (EDTA blood)
ADAMTS13 deficiency	
ADAMTS13 activity	FRETS vWF73 (citrate plasma)*
Rare HUS causes	
Defective cobalamin metabolism	
- Homocysteine levels	HLPC (potassium-EDTA plasma)*,†
- Methylmalonic acid levels	LC-Tandem MS (potassium-EDTA plasma)*,†
- Mutational screening MMACHC	Sequencing analysis (EDTA blood)
HIV	Serology
Pregnancy	Pregnancy test
HELLP syndrome	Liver enzymes
Antiphospholipid syndrome	Antiphospholipid antibody
Systemic lupus erythematosus	- Antinuclear antibody
	- Lupus anticoagulant
STEC infection	Culture, PCR, serology, anti-O157 antibody
Streptococcus pneumoniae infection	Culture, PCR, Coombs test, peanut lectin, activity test, transferrin isoelectric focussing

***Serum and EDTA plasma samples need to be centrifuged as soon as possible after sampling (preferably within 60 minutes). †For the analysis of C3d, homocysteine, and methylmalonic acid levels, (potassium) EDTA blood needs to be placed on ice immediately after sampling. ADAMTS13 = a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; CFB = complement factor B; CFH = complement factor H; CFI = complement factor I; EDTA = ethylenediaminetetraacetic acid; ELISA = enzyme-linked immunosorbent assay; FACS = fluorescent-activated cell sorting; FRETS vWF73 = fluorescence- quenching substrate for ADAMTS13; MCP = membrane cofactor protein; HELLP = haemolysis, elevated liver enzymes and low platelets; HIV = human immunodeficiency virus; HPLC = high-performance liquid chromatography; LC-Tandem MS = liquid chromatography–tandem mass spectrometry; MMACHC = methylmalonic aciduria and homocystinuria type C protein.**

Incomplete penetrance of aHUS

Mutations in complement (regulating) genes can be found in healthy family members: the penetrance of disease among carriers of mutations in *CFH*, *CFI*, and *MCP* is approximately 50 to 60%.[18,30] This indicates that the genetic aberrations are probably important for the development of aHUS, but not the sole cause. Affected patients may carry combined mutations, in more than one gene,[18,19] or carry a mutation in combination with the associated CFH or MCP haplotype. Family members who only carried one mutation or no polymorphisms were not affected,[31] but this could be due to incomplete penetrance as well. Atypical HUS may not occur until adulthood, even in patients with multiple genetic defects. This indicates that an environmental factor, such as a complement trigger, is probably needed to develop the disease. For instance, Caprioli *et al.* reported that in 77% of the patients with a mutation in *CFH*, *CFI*, or *MCP*, the clinical symptoms were preceded by flulike symptoms, gastroenteritis, or other infections.[18]

OUTCOME OF DISEASE

In about 60% of the patients with a mutation aHUS is diagnosed during childhood and in more than half of the cases, the disease is triggered by an infection or pregnancy.[18,30,32] Prognosis of patients with aHUS is poor, up to 25% of patients may not survive the acute phase and up to 50% of the patients progress to ESRD.[7,8] However, outcome of the disease is dependent on the underlying genetic aberrations. Eighty to 90% of the patients with an MCP mutation will develop a remission, although recurrences often occur.[18,30] In contrast, 60 to 70% of the patients with a *CFH*, *CFI*, or *C3* mutation will develop terminal renal failure within one year after diagnosis; in patients with αFH this amount is 30%. Not many patients with an aberration in *CFB* have been reported yet, but in 88% of the patients of one study, renal function was lost within one year after diagnosis.[30]

The underlying complement defect also determines whether therapy is needed and if it will be effective. For instance, in patients with an MCP mutation alone, plasma therapy is of limited added value: remission is achieved in 80 to 90% of these patients without plasma treatment.[30] Since MCP is a membrane-bound protein, a defect MCP protein cannot therefore be substituted by plasma therapy.

TREATMENT OPTIONS

The overall outcome of patients with aHUS is poor. To be effective, treatment must be started urgently, preferably within 24 hours after diagnosis. At this moment it takes many weeks to several months to perform the laboratory assays and genetic studies that are needed to specify the underlying cause. Furthermore, at this moment no complement abnormalities can be found in the 40% of patients. Therefore, it is advised to start plasmapheresis, which replaces missing or deficient proteins and removes disease causing antibodies, as the first treatment option. New treatment options such as complement inhibitors are now available.

Plasma therapy

Although there is no evidence from randomised controlled trials, plasmapheresis is the first-choice therapy in patients with aHUS due to a complement dysregulation. Cohort studies have shown that the mortality rate has decreased from 50 to 25% since the introduction of plasma therapy.[30] Although plasma infusion would be sufficient in patients with a missing or defective complement-regulating protein such as CFH, plasmapheresis is advised in the initial acute phase of the disease. Moreover, in the absence of a diagnosis, treatment should be directed at the removal of antibodies.

If plasmapheresis is not available or cannot be applied immediately in the acute phase, we advise to start plasma infusion, since a defective protein is the underlying cause in the majority of patients with aHUS. After the initial period of plasmapheresis, complement deactivation can often be accomplished with lesser amounts of plasma and the therapy can be switched to plasma infusion, unless the tests have demonstrated that antibodies are the cause of the disease. Obviously there are risks associated with the infusion of plasma in patients who are already hypertensive and volume overloaded due to renal impairment.

In the most recent guidelines, it is recommended to start plasmapheresis within 24 hours of diagnosis.[26,27] It is suggested to exchange 1.5 times the expected plasma volume (60 to 75 ml/kg) and replace plasma with fresh frozen plasma or virus-inactivated pooled plasma. There are no evidence-based treatment schedules for plasmapheresis treatment reported in the literature. Guidelines published by the European Paediatric Study Group on HUS[26] recommend the following: plasmapheresis should be performed daily for five days, then five sessions a week for two weeks, and then three times a week for two weeks. When plasma infusion is used instead of plasmapheresis, the suggested dosage is 30 to 40 ml/kg initially and 10 to 20 ml/kg per day thereafter.[7] The dose and frequency may be reduced to weekly or biweekly intervals if plasma therapy appears to be successful.

To monitor the response to plasma therapy, the best parameters are platelet count, and lactate dehydrogenase and haemoglobin levels in serum (haematological remission). Haptoglobin levels often remain decreased after achieving haematological remission and are therefore not used as a parameter. To determine the total treatment time, no valid parameter is available, but recommendations state that treatment should be continued for at least two days after complete remission has been achieved.[7] However, some aHUS patients will be plasma dependent and need chronic plasma treatment to stay in remission. Furthermore, it is known that in both adults and children intercurrent infections as well as vaccinations can trigger a relapse of aHUS,[32,33] for which plasmatherapy has to start again or needs to be intensified.

For patients with an MCP mutation alone, plasma therapy has limited value in the treatment of aHUS: remission is achieved in 80 to 90% of these patients without plasma treatment.[30] MCP is a membrane-bound protein and a defect MCP protein can therefore not be substituted by plasma therapy. However, since it is not known which complement genes are involved in the pathogenesis of aHUS at first presentation and since it is known that combined mutations in complement genes can occur, plasma therapy remains the first choice of treatment.

Besides plasmapheresis, avoiding triggers of endothelial injury, such as hypertension and hypercholesteraemia, by adequate blood pressure control and the use of statins are important treatment options in the acute phase of the disease and should be maintained once in remission.

Transplantation

The clinical outcome of renal transplantation in patients with aHUS is dismal. Approximately 50% of patients with aHUS will develop recurrent disease and graft loss. There are no clinical predictors of outcome, although the use of a calcineurin inhibitor after transplantation is associated with an even higher recurrence rate.[34] Unfortunately, patients with aHUS are also more prone to develop acute rejections, which also affects graft survival. Knowledge of the underlying genetic defect is helpful in predicting prognosis. The recurrence risk in patients with a *CFH* mutation is 75 to 90%; for patients with a *CFI* mutation this is 45 to 80%, and in case of a *C3* mutation, the risk of an aHUS recurrence is 40 to 70%.[35] Recurrences have been seen in patients with *CFB* and thrombomodulin mutations, as well. On the other hand, patients with a mutation in the gene encoding the membrane-bound MCP have a low risk to develop a disease recurrence in the graft.

Admittedly, it can be debated whether knowledge of the underlying genetic defect affects the management of the patient with aHUS after transplantation. Patients should be informed of the high risk of recurrence, and care should be taken to minimise endothelial injury.

Therefore, it is advised to avoid long ischaemia times, not to accept kidneys of non-heart-beating donors, and not to use calcineurin inhibitors. A recurrence should be treated with plasmapheresis, and most centres would use prophylactic plasmapheresis. Indeed plasmapheresis before and after renal transplantation has been beneficial,[35] but given the poor outcome associated with a recurrence, there is a debate on whether isolated renal transplantation should be offered to patients at high risk of a recurrence. Some authors have suggested that a combined kidney and liver transplantation with preventive plasmapheresis should be performed in patients with a known *CFH* of *CFI* mutation.[36] However, the risky procedure of a combined kidney and liver transplantation must be weighed against the estimated risk of recurrence. Hopefully, emerging therapies with complement inhibitors will allow successful kidney only transplantation in the near future (see below). Knowledge of the underlying genetic defect is critical when considering a living-related kidney transplantation. Until recently, living kidney donations were considered unjustified in patients with aHUS: there is not only a high risk of graft failure in the recipient, more importantly the donor may be a carrier of the mutation and could develop aHUS due to uncontrolled complement activation during the donor procedure.[37] If a mutation is identified in the acceptor, family members can be screened for this mutation, and only donors without this mutation can be accepted for donation. Of note, if no mutations are found, current policy is to not accept any related donor, as genetic aberrations may be present in not yet associated genes.

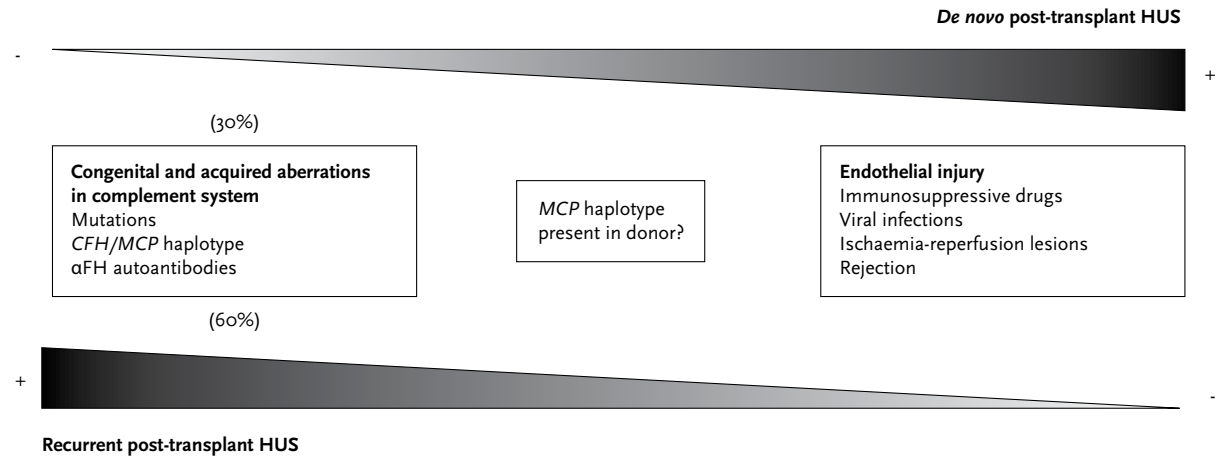
The burden of endothelial injury in a post-transplantation setting, caused for instance by immunosuppressive drugs, viral infections or rejection, might trigger *de novo* HUS in the presence of mild genetic susceptibility to HUS.[38] Possible causes of recurrent and *de novo* post-transplant HUS, both genetic and environmental, are shown in *figure 3*. Although the influence of environmental factors leading to endothelial injury is probably higher in *de novo* HUS, genetic aberrations in the complement system are still found in 30% of the patients diagnosed with *de novo* post-transplant HUS.[38] To minimise the environmental risks, adequate control of blood pressure and hypercholesterolaemia in combination with the prudent use of calcineurin inhibitors during renal transplantation is warranted (reviewed by Zuber *et al.*[38]).

Emerging new therapies

A new drug recently registered by the FDA and EMEA for the treatment of aHUS patients is the recombinant, humanised, monoclonal anti-C5 antibody eculizumab (Soliris®, Alexion Pharmaceuticals, Cheshire, CT, USA). Eculizumab specifically binds to C5, thereby blocking the cleavage of C5 into C5b (*figure 1*). In this way the formation of the anaphylatoxin C5a and of the membrane attack complex C5b-9 is prevented.

Eculizumab has been approved worldwide for the treatment of paroxysmal nocturnal haemoglobinuria (PNH), a haematological disease associated with loss of regulation of the terminal complement pathway on erythrocytes.[39] Since the first successful reports of eculizumab treatment in aHUS patients, many reports have followed, describing patients who received eculizumab to rescue their native kidneys or to prevent a recurrence in a graft after transplantation (reviewed by

Figure 3. *Genetic and environmental factors associated with post-transplant HUS*



Endothelial injury in the graft can occur due to genetic aberrations in the alternative pathway of complement system (more often in recurrent post-transplant aHUS) or by environmental factors (more often in *de novo* post-transplant HUS). The presence of the MCP_{GGAAC} haplotype, associated with the pathogenesis of aHUS, in the donor could influence a recurrence as well, although this has not been investigated extensively.

Loirat *et al.*[35] and Köse *et al.*).[40] Relapses after eculizumab treatment have only been seen when the treatment was discontinued or in patients who received a single dose; all other patients went into remission.

Two international multicentre prospective phase 2 open-label clinical trials in adolescent and adult aHUS patients and a retrospective study in children have been conducted so far.[41,42] The results showed that thrombocyte levels increased and renal function already improved from the first dose of eculizumab. None of the patients required a TMA intervention (plasmapheresis or dialysis) during the treatment. Eculizumab was well tolerated in these clinical studies. Adverse effects that were most frequently reported were hypertension, upper respiratory tract infection, and diarrhoea. In STEC-HUS patients, eculizumab is not indicated as a treatment option.

As clearance of *Neisseria meningitidis* is highly dependent on the terminal complement pathway, patients treated with eculizumab are at a higher risk for meningococcal infection. Therefore, patients should be vaccinated at least two weeks before the start of the treatment. As vaccination does not protect against all serotypes, both patients and physicians should be aware of early signs of meningococcal infection.[43] Attention also has to be paid to patients treated with immunosuppressive drugs, as these therapies can reduce the response upon vaccination.

Eculizumab is very expensive: current estimates are up to € 300,000 per treatment year. Although this drug certainly has changed the future perspectives of patients with aHUS, many unsolved questions remain: who should receive the drug, what treatment schedules should be used, and how long should therapy be continued. It is even undecided if prophylactic treatment is needed. Cost-effectiveness should be evaluated in carefully conducted prospective cohort studies.

CONCLUSIONS

The atypical haemolytic uraemic syndrome is a multigenetic and multifactorial disease associated with predisposing genetic variation in genes encoding proteins involved in regulation and activation of the alternative complement pathway. Other factors, including genetic polymorphisms, environmental factors, medication, and systemic disease, may contribute to the development of aHUS. Plasma therapy is still the first choice of treatment, but new treatment possibilities, such as the complement inhibitor eculizumab, may change this in the near future.

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REFERENCES

1. Besbas N, Karpman D, Landau D, et al. A classification of hemolytic uremic syndrome and thrombotic thrombocytopenic purpura and related disorders. Kidney Int. 2006;70(3):423-31.
2. Keir L, Coward RJ. Advances in our understanding of the pathogenesis of glomerular thrombotic microangiopathy. Pediatr Nephrol. 2011;26(4):523-33.
3. Noris M, Remuzzi G. Hemolytic uremic syndrome. J Am Soc Nephrol. 2005;16(4):1035-50.
4. Garg AX, Suri RS, Barrowman N, et al. The long-term renal prognosis of diarrhea associated hemolytic uremic syndrome: A systematic review, meta-analysis and meta-regression of 3476 children from 49 studies. J Am Soc Nephrol. 2003;14:299a-a.
5. Frank C, Werber D, Cramer JP, et al. Epidemic profile of Shiga-toxin-producing Escherichia coli O104:H4 outbreak in Germany. N Engl J Med. 2011;365(19):1771-80.
6. Kemper MJ. Outbreak of hemolytic uremic syndrome caused by E. coli O104:H4 in Germany: a pediatric perspective. Pediatr Nephrol. 2012;27(2):161-4.
7. Kavanagh D, Goodship TH, Richards A. Atypical haemolytic uraemic syndrome. Br Med Bull. 2006;77-78:5-22.
8. Jokiranta TS, Zipfel PF, Fremeaux-Bacchi V, Taylor CM, Goodship TJ, Noris M. Where next with atypical hemolytic uremic syndrome? Mol Immunol. 2007;44(16):3889-900.
9. Tsai HM, Chandler WL, Sarode R, et al. Von Willebrand factor and von Willebrand factor-cleaving metalloprotease activity in Escherichia coli O157 : H7-associated hemolytic uremic syndrome. Pediatr Res. 2001;49(5):653-9.
10. Mannucci PM, Franchini M. Advantages and limits of ADAMTS13 testing in the prognostic assessment of thrombotic thrombocytopenic purpura. Presse Med. 2012.
11. Cameron JS, Vick R. Letter: Plasma-C3 in haemolytic-uraemic syndrome and thrombotic thrombocytopenic purpura. Lancet. 1973;2(7835):975.
12. Monnens L, Molenaar J, Lambert PH, Proesmans W, van Munster P. The complement system in hemolytic-uremic syndrome in childhood. Clin Nephrol. 1980;13(4):168-71.
13. Walport MJ. Complement. First of two parts. N Engl J Med. 2001;344(14):1058-66.
14. Walport MJ. Complement. Second of two parts. N Engl J Med. 2001;344(15):1140-4.
15. Warwicker P, Goodship THJ, Donne RL, et al. Genetic studies into inherited and sporadic hemolytic uremic syndrome. Kidney Int. 1998;53(4):836-44.
16. Noris M, Brioschi S, Caprioli J, et al. Familial haemolytic uraemic syndrome and an MCP mutation. Lancet. 2003;362(9395):1542-7.
17. Fremeaux-Bacchi V, Dragon-Durey MA, Blouin J, et al. Complement factor I: a susceptibility gene for atypical haemolytic uraemic syndrome. J Med Genet. 2004;41(6):e84.

18. Caprioli J, Noris M, Brioschi S, et al. Genetics of HUS: the impact of MCP, CFH, and IF mutations on clinical presentation, response to treatment, and outcome. Blood. 2006;108(4):1267-79.

19. Westra D, Volokhina E, van der Heijden E, et al. Genetic disorders in complement (regulating) genes in patients with atypical haemolytic uraemic syndrome (aHUS). Nephrol Dial Transplant. 2010;25(7):2195-202.

20. Delvaeye M, Noris M, De Vriese A, et al. Thrombomodulin mutations in atypical hemolytic-uremic syndrome. N Engl J Med. 2009;361(4):345-57.

21. De Goicoechea JE, Harris CL, Esparza-Gordillo J, et al. Gain-of-function mutations in complement factor B are associated with atypical hemolytic uremic syndrome. Proc Natl Acad Sci U S A. 2007;104(1):240-5.

22. Fremeaux-Bacchi V, Miller EC, Liszewski MK, et al. Mutations in complement C3 predispose to development of atypical hemolytic uremic syndrome. Blood. 2008;112(13):4948-52.

23. Esparza-Gordillo J, Goicoechea de Jorge E, Buil A, et al. Predisposition to atypical hemolytic uremic syndrome involves the concurrence of different susceptibility alleles in the regulators of complement activation gene cluster in 1q32. Hum Mol Genet. 2005;14(5):703-12.

24. Jozsi M, Licht C, Strobel S, et al. Factor H autoantibodies in atypical hemolytic uremic syndrome correlate with CFHR1/CFHR3 deficiency. Blood. 2008;111(3):1512-4.

25. Jozsi M, Strobel S, Dahse HM, et al. Anti factor H autoantibodies block C-terminal recognition function of factor H in hemolytic uremic syndrome. Blood. 2007;110(5):1516-8.

26. Ariceta G, Besbas N, Johnson S, et al. Guideline for the investigation and initial therapy of diarrhea-negative hemolytic uremic syndrome. Pediatr Nephrol. 2009;24(4):687-96.

27. Taylor CM, Machin S, Wigmore SJ, Goodship TH. Clinical practice guidelines for the management of atypical haemolytic uraemic syndrome in the United Kingdom. Br J Haematol. 2010;148(1):37-47.

28. Saunders RE, Goodship TH, Zipfel PF, Perkins SJ. An interactive web database of factor H-associated hemolytic uremic syndrome mutations: insights into the structural consequences of disease-associated mutations. Hum Mutat. 2006;27(1):21-30.

29. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing Escherichia coli and haemolytic uraemic syndrome. Lancet. 2005;365(9464):1073-86.

30. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. N Engl J Med. 2009;361(17):1676-87.

31. Caprioli J, Castelletti F, Bucchioni S, et al. Complement factor H mutations and gene polymorphisms in haemolytic uraemic syndrome: the C-257T, the A2089G and the G2881T polymorphisms are strongly associated with the disease. Hum Mol Genet. 2003;12(24):3385-95.

32. Geerdink LM, Westra D, van Wijk JAE, et al. Atypical hemolytic uremic syndrome in children: complement mutations and clinical characteristics. Pediatr Nephrol. 2012 Mar 13.

33. Olie KH, Goodship THJ, Verlaak R, et al. Posttransplantation cytomegalovirus-induced recurrence of atypical hemolytic uremic syndrome associated with a factor H mutation: Successful treatment with intensive plasma exchanges and ganciclovir. Am J Kidney Dis. 2005;45(1):E12-E5.

34. Artz MA, Steenbergen EJ, Hoitsma AJ, Monnens LAH, Wetzels JFM. Renal transplantation in patients with hemolytic uremic syndrome: High rate of recurrence increased incidence of acute rejections. Transplantation. 2003;76(5):821-6.

35. Loirat C, Fremeaux-Bacchi V. Atypical hemolytic uremic syndrome. Orphanet J Rare Dis. 2011;6(1):60.

36. Davin JC, Strain L, Goodship THJ. Plasma therapy in atypical haemolytic uremic syndrome: lessons from a family with a factor H mutation. Pediatr Nephrol. 2008;23(9):1517-21.

37. Donne RL, Abbs I, Barany P, et al. Recurrence of hemolytic uremic syndrome after live related renal transplantation associated with subsequent de novo disease in the donor. Am J Kidney Dis. 2002;40(6):E22.

38. Zuber J, Le Quintrec M, Sberro-Soussan R, Loirat C, Fremeaux-Bacchi V, Legendre C. New insights into postrenal transplant hemolytic uremic syndrome. Nature Rev Nephrol. 2011;7(1):23-35.

39. Hillmen P, Hall C, Marsh JC, et al. Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. N Engl J Med. 2004;350(6):552-9.

40. Kose O, Zimmerhackl LB, Jungraithmayr T, Mache C, Nurnberger J. New treatment options for atypical hemolytic uremic syndrome with the complement inhibitor eculizumab. Semin Thromb Hemost. 2010;36(6):669-72.

41. Legendre CM, Babu S, Furman RR, et al. Safety and efficacy of eculizumab in aHUS patients resistant to plasma therapy: interim analysis from a phase II trial (SA-FC406) [abstract]. J Am Soc Nephrol. 2010;21:93A.

42. Muus P, Legendre C, Douglas K, et al. Safety and efficacy of eculizumab in aHUS patients on chronic plasma therapy: interim analysis of a phase II trial (F-PO1274) [abstract]. J Am Soc Nephrol. 2010;21:402A.

43. Bouts A, Monnens L, Davin JC, Struijk G, Spanjaard L. Insufficient protection by Neisseria meningitidis vaccination alone during eculizumab therapy. Pediatr Nephrol. 2011;26(10):1919-20.

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ORIGINAL ARTICLE

Common alternative diagnoses in general practice when deep venous thrombosis is excluded

A.J. ten Cate-Hoek[1]*, E.F. van der Velde[2], D.B. Toll[3], H.C.P.M. van Weert[2], K.G.M. Moons[3], H.R. Büller[2], A.W. Hoes[3], M.A. Joore[1], R. Oudega[3], M.H. Prins[1], H.E.J.H. Stoffers[1]

[1]Maastricht University, School of Public Health and Primary Care (CAPHRI), Departments of Epidemiology and General Practice, Academic Hospital Maastricht, Departments of Clinical Epidemiology and Medical Technology Assessment and Internal Medicine, Maastricht, the Netherlands, [2]Departments of Vascular Medicine and General Practice, Academic Medical Center, University of Amsterdam, the Netherlands, [3]University Medical Center Utrecht, Julius Center for Health Sciences and Primary Care, Utrecht, the Netherlands, *corresponding author: tel. +31 (0)43-3871243, e-mail: arina.tencate@epid.unimaas.nl, arina.cate@mumc.nl

ABSTRACT

Background: In patients initially suspected of deep venous thrombosis (DVT) the diagnosis can be confirmed in approximately 10 to 30% of cases. For the majority of patients this means that eventually an alternative diagnosis is assigned.

Objective: To assess the frequency distribution of alternative diagnoses and subsequent management of patients in primary care after initial exclusion of DVT. In addition, assess the value of ultrasound examination for the allocation of alternative diagnoses.

Methods: Data were recorded by general practitioners alongside a diagnostic study in primary care in the Netherlands (AMUSE). Additional data were retrieved from a three-month follow-up questionnaire. A descriptive analysis was performed using these combined data.

Results: The most prevalent diagnoses were muscle rupture (18.5%), chronic venous insufficiency (CVI) (14.6%), erysipelas/cellulitis (12.6%) and superficial venous thrombosis (SVT) (10.9%). Alternative diagnoses were based mainly on physical examination; ultrasound examination (US) did not improve the diagnostic yield for the allocation of alternative diagnoses. In about 30% of all cases, a wait and see approach was used (27 to 41%). During the three-month follow-up nine patients were diagnosed with venous thromboembolic disease, three of which occurred in patients with the working diagnosis of SVT (p=0.026).

Conclusions: We found that after exclusion of DVT in general practice a wait and see policy in the primary care setting is uneventful for almost one third of patients, but with the alternative diagnosis of SVT, patients may require closer surveillance since we found a significant association with thrombosis in these patients.

KEYWORDS

Deep venous thrombosis, alternative diagnoses, general practice

INTRODUCTION

The clinical diagnosis of deep venous thrombosis (DVT) is challenging. Unilateral complaints such as painful swelling of the leg, with or without accompanying redness, are common symptoms of many conditions besides DVT. This may explain why only a small proportion of patients who are clinically suspected of having a DVT and who are subsequently evaluated by ultrasound examination do actually have the condition, with rates ranging between 10 to 30% for different populations.[1-4] Therefore an alternative diagnosis is eventually assigned for the majority of patients. In order to study the distribution of alternative diagnoses in patients in whom DVT was excluded, we gathered data alongside a diagnostic management study in general practice.[5] We assessed the frequencies of alternative

diagnoses that were allocated and assessed which signs and/or symptoms are used to arrive at the different alternative diagnoses. In addition we looked at the associated disease management in these patients.
It has been suggested that the reduction of the number of ultrasound examinations by the use of a clinical decision rule in combination with a D-dimer assay and subsequent avoidance of objective testing in 30 to 50% of patients who present with complaints suspected of DVT is undesirable since these examinations could be helpful to establish an alternative diagnosis in the absence of DVT.[6]
Therefore, our study aims were to assess the frequency of common alternative diagnoses and associated disease management, to describe the signs and symptoms that were used to establish the different alternative diagnoses, and to explore the additional value of ultrasound examination for the allocation of alternative diagnoses.

METHODS

We studied the alternative diagnoses and the management of patients suspected of DVT in primary care, after DVT was ruled out based on a clinical decision strategy. From March 2005 till January 2007 data were collected alongside the 'Amsterdam Maastricht Utrecht Study on venous thrombo-Embolism' (AMUSE), a diagnostic management study in primary care.[5] We performed a descriptive analysis using these data. Briefly, the AMUSE study is a management study conducted in primary care in three regions of the Netherlands, involving 1028 consecutive patients with complaints suspect for DVT. In AMUSE the safety and efficiency of the use of a clinical decision rule including a point of care D-dimer assay to exclude DVT in primary care was evaluated. Patients who scored above a predefined threshold were referred for ultrasound; patients below the threshold were not referred.[7] Following the initial presentation, all patients were evaluated at day 7±2 by the general practitioner. For the present descriptive study, data on clinical status that were collected at this one-week follow-up visit during the management study and data derived from a questionnaire on health problems that were experienced during a period of three months after the initial presentation were analysed.

Data collection

The variables for the analysis were derived from a systematic case record form (CRF) filled out by the general practitioner at the second patient visit, after one week. The following items were recorded: leg complaints, onset of complaints, medical history, risk factors for venous thrombosis, medication use, findings at physical examination, ultrasound result (if ultrasound was conducted), result of clinical decision rule, most likely diagnosis (working diagnosis) and the therapy that was instituted.
In addition, variables were derived from questionnaires that were sent out to all patients three months after inclusion. The questionnaires comprised questions on leg complaints, office visits in general practice and/or visits to hospital-based specialists for leg problems, as well as questions on initiated therapy for these problems. All patients who did not respond (30%) were contacted. On suspicion of a (recurrent) thromboembolic event, based on the questionnaires, additional medical information was retrieved from the medical records of the treating physician.

Venous thromboembolism

Of the 1028 eligible patients available for analysis, 127 patients were diagnosed with DVT at presentation. During three months of follow-up venous thromboembolism furthermore occurred in 11 patients who were initially not diagnosed with DVT (1.1%). In total 138 patients were documented to have thrombotic disease. During the entire follow-up period ten patients died (seven were non-VTE deaths) and three patients were lost to follow-up.

Of the 1028 patients analysed, 1002 were initially assessed according to the AMUSE protocol.[5] The diagnostic clinical decision rule according to the AMUSE protocol used the following dichotomous variables: male gender, use of hormonal contraceptives, active malignancy in the past six months, surgery in the previous month, absence of leg trauma, distension of collateral veins, difference in calf circumference ≥3 cm, and D-dimer (Simplify®) abnormal.[7] Twenty-six patients were not assessed in accordance with the AMUSE protocol; they all underwent ultrasound assessment. All adverse outcome events during follow-up were assessed by an independent adjudication committee.

Alternative diagnoses

We analysed the prevalence of different alternative diagnoses as recorded by the general practitioners during the one-week follow-up visit, thus after results of the ultrasound examinations were known for referred patients. The general practitioner could choose from a list of ten possible alternative diagnoses to be recorded on the CRF: deep vein thrombosis (DVT), erysipelas /cellulitis, chronic venous insufficiency (CVI), lymphoedema, superficial venous thrombosis (SVT), mycosis, muscle rupture/haematoma, Baker's (popliteal) cyst, ankle arthritis and pelvic tumour. The general practitioners were asked to record the most likely diagnosis, in their opinion. If this diagnosis was not among these ten pre-specified diagnoses, they could record the preferred diagnosis as open text. The diagnosis of DVT could be assigned when the patient had a documented DVT

on ultrasound one week earlier or when the diagnosis of DVT was still the most likely diagnosis at that point in time, according to the general practitioner.

Statistical analysis

We first assessed the frequency of DVT and the frequencies of all alternative diagnoses in our study population. Then, for each of the four most frequent alternative (i.e. non-DVT) diagnoses, the presence of signs and symptoms was described and the association of signs and symptoms with each diagnosis was estimated. Similarly, known risk factors for thrombosis, data on ultrasound examination, therapeutic strategies and referral and follow-up practices were analysed for frequency and their respective association with each alternative diagnosis.

Associations were described as odds ratios and their 95% confidence intervals and were calculated comparing persons with a specific diagnosis (erysipelas/cellulitis, muscle rupture/haematoma, CVI, SVT, DVT, respectively) with all of those without that particular diagnosis, using univariate logistic regression and tested using the Chi-square test for categorical variables (SPSS 17.0 for Windows). A p-value ≤0.05 was considered statistically significant.

RESULTS

Common alternative diagnoses

At the one-week follow-up visit, 669/1028 CRFs (65.1%) contained a specific diagnosis. No specific diagnosis was stated on the remaining 359/1028 CRFs (34.9%). At the time of the follow-up visit 129/1028 patients had already been diagnosed with DVT. For 57/129 of these patients no diagnosis was stated on the CRF, leaving 302/1028 (29%) of patients without an alternative diagnosis at week 1. Although 29% of cases did not have a diagnosis stated at the one-week visit, all but three of the 1028 patients were followed up. Among the diagnoses given, the four most prevalent alternative diagnoses to DVT were muscle rupture/haematoma (18.5%), CVI (14.6%), erysipelas/cellulitis (12.6%), and SVT (10.9%). The other alternative diagnoses mentioned were each present in less than 6% (lymphoedema (5.5%), Baker's (popliteal) cyst (4.5%), pelvic tumour (0.6%), ankle arthritis (1.8%), and mycosis (0%)). Fifteen percent of the general practitioners described an alternative diagnosis other than the ten diagnostic options mentioned in the CRF. The added alternatives were mainly described as: muscle complaints, lower back hernia, known oedema and gonarthrosis.

Table 1. *Signs and symptoms for each alternative diagnosis besides DVT*

Disease	**Erysipelas/cellulitis N = 84**		**Muscle rupture/ hematoma N = 124**		**CVI N = 98**		**SVT N = 73**		**DVT N = 138**	
	N (%)	P OR (95% CI)	N (%)	P OR (95% CI)	N (%)	P OR (95% CI)	N (%)	P OR (95% CI)	N (%)	P OR (95% CI)
Signs, symptoms										
Right side	38(49)		56(49)		48(53)		49		56(42)	
Pain	76(92)		113(92)	< 0.05 OR=2.1 (1.04-4.03)	71(76)	<0.01 OR=0.4 (0.25-0.7)	69(95)	<0.05 OR=3.1 (1.1-8.3)	118(86)	
Swelling	80(96)	<0.001 OR=8.6 (2.8-26.1)	86(69)	<0.01 OR=0.6 (0.36-0.86)	80(84)		51(70)		126(91)	<0.001 OR=3.4 (1.9-6.2)
Swelling entire leg	15(19)		18(15)	<0.05 OR=0.2 (0.09-0.29)	30(32)	0.01 OR=1.9 (1.2-3.03)	9(13)	0.05 OR=0.5 (0.24-1.0)	48(35)	<0.001 OR=2.1 (1.5-3.2)
Painful palpation vein	39(48)		67(57)		49(47)		57(80)	<0.001 OR=3.7 (2.0-6.7)	69(53)	
Redness	76(91)	<0.001 OR=21.3 (10.2-44.3)	15(12)	<0.001 OR=0.6 (0.34-0.99)	30(33)		37(51)	<0.05 OR=1.8 (1.1-2.8)	53(40)	
Collateral vein dilatation	10(26)		11(9)		20 (21)		19(26)		29(21)	<0.05 OR=1.7 (1.1-2.6)
Acute onset	48(61)		71(59)		34(38)	<0.01 OR=0.5 (0.34-0.86)	40(56)		76(57)	

P values concern the comparison of patients with single diagnosis present versus all other patients (diagnosis absent) and are only presented if <0.05. Then, odds ratios and 95% confidence intervals are included as well (n = 669).

Muscle rupture/haematoma was the only diagnosis that was seen equally frequently in men and women, whereas the other alternative diagnoses occurred significantly more often in women (p≤0.05).

Association with signs and symptoms

Table 1 shows the distribution of signs and symptoms for each of the four most frequent alternative diagnoses and DVT respectively. 'Pain' and 'swelling' were common findings in all four most frequent alternative diagnoses. For most of the alternative diagnoses the onset of complaints was acute. An exception was the non-acute onset of CVI in a majority (61.8%) of cases: OR 0.5 (0.34 to 0.86). Compared with the other alternative diagnoses, CVI also presented more often with 'swelling of the entire leg'. 'Redness' was the most distinctive feature of erysipelas: OR 21.3 (10.2 to 44.3) and was present in 91% of these patients. In contrast, 'redness' as well as 'swelling' was more often absent in muscle rupture/haematoma. 'Painful palpation of the vein was observed to be discriminatory for SVT: OR 3.7 (1.1 to 8.3).

Association with risk factors for thrombosis

The distribution of classic risk factors for venous thromboembolism of each alternative diagnosis was compared with the distribution of these risk factors in patients with confirmed DVT (*table 2*). Muscle rupture/haematoma was negatively associated with most risk factors for thrombosis. In contrast, positive associations with previous SVT (OR 2.9 (1.7 to 5.1)), and previous DVT (OR 5.2 (2.8 to 9.5)) were observed for SVT. CVI showed positive associations with active malignancy (OR 2.4 (1.6 to 3.8)) and malignancy not active (OR 2.1(1.0 to 4.4)).

Therapeutic strategies

The instituted therapy as recorded in the CRF at week 1 was analysed for the four most prevalent alternative

Table 2. *Risk factors for each alternative diagnosis besides DVT*

Disease	Erysipelas/cellulitis N = 84		Muscle rupture/ hematoma N = 124		CVI N = 98		SVT N = 73		DVT N = 138	
	N (%)	P OR (95% CI)	N (%)	P OR (95% CI)	N (%)	P OR (95% CI)	N (%)	P OR (95% CI)	N (%)	P OR (95% CI)
Risk factors										
Malignancy active	2(2)		2(2)	<0.05 OR=0.25 (0.1-0.9)	10(10)	<0.05 OR=2.4 (1.6-3.8)	4(6)		15(11)	<0.01 OR=2.6 (1.4-4.9)
Malignancy not active	4(5)		4(3)		10(10)	<0.05 OR=2.1 (1.0-4.4)	5(7)		14(10)	<0.05 OR=1.9 (1.0-3.4)
Age > 70 yr	28(35)		22(19)	0.001 OR=0.4 (0.27-0.7)	42(44)	<0.01 OR=2 (1.3-3.1)	27(38)		39(29)	
Previous DVT	5(7)	<0.05 OR=0.3 (0.1-0.8)	13(15)		19 (20)		22(31)	0.001 OR=2.9 (1.7-5.1)	30(22)	<0.05 OR=1.6 (1.1-2.6)
Previous SVT	8(10)		4(3)	< 0.05 OR=0.3 (0.12-0.8)	7(7)		19(26)	<0.001 OR=5.2 (2.8-9.5)	9(7)	
Recent surgery	3(4)		4(3)	<0.05 OR=0.4 (0.1-0.98)	11(11)		3(4)		11(8)	
Travel	6(8)		13(11)		7(7)		8(11)		20(15)	<0.05 OR=1.9 (1.1-3.3)
Trauma absent	59(70)		77(62)	<0.01 OR=0.5 (0.34-0.8)	77(79)		58(79)		109(79)	
Male gender	37(44)		56(45)		30(31)	<0.05 OR=0.7 (0.4-1.1)	19(26)	<0.05 OR=0.5 (0.3-0.9)	69(50)	<0.001 OR=1.9 (1.3-2.7)
Varicositas	24(29)		27(22)	<0.001 OR=0.4 (0.27-0.7)	51(55)	<0.001 OR=2.4 (1.6-3.8)	47(67)	<0.001 OR=4.2 (2.5-7.1)	44(33)	

P values concern the comparison of patients with single diagnosis present versus all other patients (diagnosis absent) and are only presented if <0.05. Then, odds ratios and 95% confidence intervals are included as well (n = 669).

diagnoses. The preprinted therapeutic options on the CRF that the general practitioner could choose from where: antibiotic therapy, pain reduction (NSAIDs), antithrombotic therapy (LMWH and coumarins), compression therapy, physiotherapy or no therapy (observant).

In almost 30% of cases, a wait and see approach was used and no therapy was instituted (27 to 41%). For the diagnosis of muscle rupture/haematoma a wait and see policy was significantly more often followed than for the other alternatives. In contrast, for the diagnosis of erysipelas/cellulitis no immediate action was undertaken in only 7% of the cases; in 70% antibiotic therapy was instituted, which was combined with compression therapy in a third of the cases (35%).

For CVI, compression therapy was the most common form of therapy (33%) when therapy was stipulated, and furthermore diuretics (10%) as well as NSAIDs (7%) were prescribed. In the treatment of SVT, compression therapy was observed to be the main therapeutic feature (40%). The four additional forms of therapy were NSAIDs, LMWH, coumarins and antibiotics (19%, 12%, 7% and 6% respectively).

Referral and follow-up

The majority of patients with an alternative diagnosis were followed up in general practice. Patients were referred for further evaluation and therapy in secondary care in 15 to 20% of cases. Of the patients with the diagnosis of muscle rupture/haematoma and CVI 10% and 9%, respectively, were referred to the surgical department for evaluation. Patients with the diagnosis of SVT and erysipelas were most commonly referred to a dermatologist (9 to 11%).

On average, patients visited their general practitioner in the follow-up of leg complaints two to three times (mean 2.4, SD 3.7) over the course of three months.

For most alternative diagnoses the maximum number of visits was six.

Additional value of ultrasound examination

Ultrasound examination was only performed in patients with either a high clinical score and/or a positive result on D-dimer testing. The avoidance of ultrasound examinations in 50% of patients suspected of DVT did not, however, impact the prevalence of the most common alternative diagnoses. The same distribution was found for SVT and erysipelas irrespective of ultrasound examination: SVT without ultrasound in 9.2% (32/349) *vs* 12.8% (41/320) with ultrasound, p=0.13; erysipelas without ultrasound in 12.6% (44/349) *vs* 12.5% (40/320) with ultrasound, p=0.97. Both the diagnosis of muscle rupture and CVI were more frequent in patients who did not undergo ultrasound examination 24.6% (86/349) *vs* 11.9% (38/320), p<0.0001 and 16.9 (59/349) *vs* 12.2% (39/320), p=0.09, respectively. For the less frequently observed alternative diagnoses, arthritis of the ankle was significantly more often diagnosed in patients not undergoing ultrasound: 3.3% (11/341) *vs* 0.3% (1/327), p=0.004. Also lymphoedema was significantly more often diagnosed in patients who did not have an ultrasound examination: 7.2% (24/309) *vs* 3.6% (12/321), p=0.04. This was also the case for the diagnosis of Baker's cyst: 32.7% (86/263) *vs* 11.9% (38/320), p<0.001.

Thrombotic events in relation to alternative diagnosis

During the three months follow-up period 11 patients (1.1%) who were not diagnosed with DVT at presentation (7 in the low-score group, 4 in the high-score group) were diagnosed with venous thromboembolic disease, one of which was fatal. Two of these 11 patients were diagnosed with DVT within two days after presentation; the alternative diagnoses of the remaining nine patients were SVT (3), muscle rupture (1), lymphoedema (1) and other (1). For three patients, two in the low-score group and one in the high-score group, no alternative diagnosis was stated; these patients were eventually diagnosed with pulmonary embolism.

DISCUSSION

The alternative diagnoses given were based mainly on clinical features. No specific diagnosis was stated in 29% of patients; this percentage of patients without a diagnosis is in accordance with a report from secondary care, where all patients underwent ultrasound and no diagnosis was affirmed in 24% of patients.[6] The use of a clinical decision rule and the associated 50% reduction of ultrasound examinations, therefore, are not likely accountable for the lack of allocated alternative diagnoses. Ultrasound did not improve the diagnostic yield; none of the alternative diagnoses were more prevalent among patients who did have ultrasound examination. After the exclusion of DVT a wait and see policy in the primary care setting was sufficient in one third of the patients. However, patients diagnosed as SVT may require closer surveillance because, of the patients who were diagnosed with DVT during the follow-up period of three months, three out of six were diagnosed as SVT. Although the analyses were not pre-specified it is statistically unusual (p=0.026) that of the nine missed venous thromboembolic events, three occurred in patients with the working diagnosis of SVT. None of these patients were treated with anticoagulant medication. The omission of treatment could very well be influenced by lack of information on the extent of the thrombus into the (deep) venous system. SVT has been reported in association with DVT in several instances, a review of cases of SVT in primary care showed that DVT occurred in 2.7% of all SVT patients as compared with 0.2% in the controls: OR=10.2

(2.0-51.6).[8,9] A recently published retrospective evaluation of therapeutic management and clinical outcome of SVT in a secondary care facility[10] also showed that SVT may be prone to venous thromboembolism. By using a clinical decision rule in combination with D-dimer testing the extra information retrieved from D-dimer testing may also guide the decision process towards the allocation of an alternative diagnosis. SVT was both significantly associated with a positive result on D-dimer testing and with a more prothrombotic profile.

Some limitations of this study have to be mentioned. First, all alternative diagnoses in our cohort were working diagnoses; no objective diagnostic testing was performed to confirm these diagnoses. For most diagnoses, however, no gold standard diagnostic tests are available or routinely used if available; in our opinion, this study is therefore a good representation of current practice. Second, for 29% of patients no alternative diagnosis was stated; all of these patients, however, had a follow-up without incident.

In conclusion: We found that the reduction in the number of ultrasound examinations does not influence the diagnostic yield and that after exclusion of DVT based on a decision strategy a wait and see policy in the primary care setting is uneventful for almost one third of patients. Patients with the diagnosis of SVT may require closer surveillance since we found a significant association with thrombosis in these patients.

ACKNOWLEDGEMENTS

We gratefully acknowledge the enthusiastic participation of general practitioners and other health care professionals who contributed to the study.

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REFERENCES

1. Cogo A, Lensing AWA, Koopman MMW, et al. Compression ultrasonography for diagnostic management of patients with clinically suspected deep vein thrombosis.: prospective cohort study. BMJ. 1998;316:17-20.
2. Lensing AW, Prandoni P, Prins MH, Buller HR. Deep-vein thrombosis. Lancet. 1999;353:479-85.
3. Ten Cate-Hoek AJ, Prins MH. Management studies using a combination of D-dimer test result and clinical probability to rule out venous thromboembolism: a systematic review. J Thromb Haemost. 2005;3:2465-70.
4. Wells PS, Owen C, Doucette S, et al. Does this patient have deep vein thrombosis? JAMA. 2006;295:199-207.
5. Buller HR, ten Cate-Hoek AJ, Hoes AW, et al. Safely Ruling Out Deep Venous Thrombosis in Primary Care. Ann Intern Med. 2009;150:229-35.
6. Blättler W, Martinez I, Blättler IK. Diagnosis of deep venous thrombosis and alternative diseases in symptomatic outpatients. Eur J Intern Med. 2004;15:305-11.
7. Oudega R, Moons KG, Hoes AW. Ruling out deep venous thrombosis in primary care. A simple diagnostic algorithm including D-dimer testing. Thromb Haemost. 2005;94:200-5.
8. Di Nisio M, Wichers IM, Middeldorp S. Treatment of superficial venous thrombosis of the leg (review). The Cochrane Library 2008, issue 1.
9. van Weert H, Dolan G, Wichers I, et al. Spontaneous superficial venous thrombophlebitis: Does it increase risk for thromboembolism? A historic follow-up study in primary care. J Fam Pract. 2006;55:52-7.
10. Wichers IM, Haighton M, Büller HR, Middeldorp S. A retrospective analysis of patients treated for superficial vein thrombosis. Neth J Med. 2008;66:423-7.

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CASE REPORT

Isolated elevated aspartate aminotransferase: a surprising outcome for clinicians

R.R.L. Wener[1]*, F.J. Loupatty[2], W.E.M. Schouten[1]

Departments of [1]Internal Medicine, [2]Clinical Chemistry, Onze Lieve Vrouwe Gasthuis, Amsterdam, the Netherlands, *corresponding author: e-mail: rrlwener@gmail.com

ABSTRACT

In this report a case of macro-aspartate aminotransferase in a 34-year-old pregnant woman is presented. Awareness of the existence of a macroenzyme is important because of their ability to cause diagnostic confusion, which leads to unnecessary investigations. Confirmation with a polyethylene glycol precipitation test is simple to perform and not expensive.

KEYWORDS

Macro-enzyme, macro-ASAT, polyethylene glycol precipitation

INTRODUCTION

Although increased enzyme activity in serum is usually associated with disease, benign conditions have been described in which serum enzymes are abnormal. Indeed, several enzymes can form high-molecular-mass complexes either by self-polymerisation or by association with other plasma components, i.e. immunoglobulins.[1,2] This phenomenon was first recognised in 1964.[3] Although serum activity may be unaffected, in some cases, immunoglobulin binding to circulating enzymes may lead to an increased activity by mechanisms involving reduced inactivation, clearance or excretion.[1] Unfortunately, routine laboratory analysis is inapt to differentiate between enzyme activity and a corresponding macroenzyme species. As a result, the presence of a macroenzyme may cause diagnostic confusion if remained undetected. Here, a case of a persistent and isolated elevation of aspartate aminotransferase (ASAT) activity in serum due to a macroenzyme of ASAT is presented. This report illustrates the importance of recognising macroenzyme species and clear documentation of this biochemical abnormality to prevent misinterpretation and to avoid excessive investigations.

CASE REPORT

A 34-year old Polish woman (G2P0), who was 34 weeks pregnant, presented with pain in the right upper abdomen. Her medical history revealed severe abdominal and pelvic trauma, nephrolithiasis, benign ovarian cyst, haematocolpos and pregnancy after an intracytoplasmic sperm injection procedure. She denied fever, jaundice, muscle pain or weakness. She occasionally took an antacid. Full physical examination was normal, including a normal pregnancy. However, laboratory examination demonstrated a persistent, isolated elevation of ASAT activity (397 IU/l; 371 IU/l, reference interval ASAT <30 IU/l). Additional laboratory findings included: haemoglobin 7.6 mmol/l (7.5 to 10 mmol/l), erythrocytes 3.9 x 10^{12}/l (4.0 to 5.0 x 10^{12}/l), white blood cell count 14.9 x 10^9/l (4 to 10 x 10^9/l), platelets 318 x 10^9/l (150 to 400 x 10^9/l), creatinine 44 µmol/l (50 to 95 µmol/l), lactate dehydrogenase 174 IU/l (<250 IU/l), creatine kinase 58 IU/l (<45 IU/l), bilirubin 5 µmol/l (<17 µmol/l), and haptoglobin 1.15 g/l (0.40 to 2.45). Imaging studies demonstrated no abnormalities of the liver, pancreas, gallbladder and kidneys. Normal values for alanine aminotransferase and gamma glutamyltransferase made hepatic disease very unlikely. Acute viral hepatitis was excluded by serological measurements for hepatitis B and C, Epstein-Barr virus and cytomegalovirus. No evidence for other sources of ASAT, such as myocardial disease, skeletal muscle disorders or haemolysis, was found. The patient's abdominal pain spontaneously resolved and the patient became asymptomatic. At this point, the presence of macro-ASAT was suggested by the

clinical chemist. ASAT activity was determined in the human plasma of our patient and two control subjects before and after treatment with polyethylene glycol as described previously.[4] The results are shown in *table 1*. Our patient demonstrated that 98% ASAT activity was precipitated with polyethylene glycol, whereas two controls showed 24 and 37% PPA (polyethylene glycol-precipitable activity, reference values 18 to 53% PPA),[4] confirming the presence of macro-ASAT.

DISCUSSION

Aspartate aminotransferase is present in significant amounts in the liver, heart, skeletal muscle and erythrocytes. Injury to any of these organs or cells can result in the release of the enzyme into the circulation. Consequently, increased serum activities of ASAT should prompt clinical evaluation, which may include abdominal imaging studies and laboratory assessment of hepatocellular, muscular, or cardiac causes. Furthermore, several types of medication (i.e. erythromycin) can cause solitarily elevated serum ASAT.[5] As mentioned earlier, our patient was not on any medication and no evidence of hepatic disease, skeletal muscle disorders or myocardial disease was found in our patient. Hence, in the absence of disease, analytical interferences should be an intrinsic part of the differential diagnosis.

Measurement of ASAT activity is clearly affected by haemolysed samples as erythrocytes contain ASAT activity up to 20 times greater than normal serum. In our case, however, lactate dehydrogenase activity, which is also abundantly present in erythrocytes, was within its reference range. In addition, measurement of the serum haptoglobin level was not decreased, excluding haemolysis as analytical interference.

Our experiments with polyethylene glycol, however, clearly demonstrated the presence of a macroenzyme species for ASAT. Until now, several cases of macro-ASAT have been reported in the literature, including apparently healthy individuals with ASAT activities as high as 50 times the upper limit of the reference range.[6-8] Although some patients have been described with various conditions, including malignancies and autoimmune disorders, the majority of reported cases are asymptomatic. Indeed, the absence of pathology over a long period of time in healthy individuals with macro-ASAT argues for the benign nature of this phenomenon.[6,9] Remarkably, the presence of macro-ASAT is not a transient phenomenon. In addition to our patient, who already demonstrated an isolated increase in ASAT in 2004, numerous cases have been reported in which macro-ASAT was persistently present for more than ten years.[6,9] Hence, it is imperative to document this essential information in the patient's medical records to avoid diagnostic confusion, perhaps years in the future.

Laboratory test results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings. However, an important clue to the presence of a macroenzyme species is the persistent elevation of a single enzyme activity in serum. Indeed, our patient demonstrated persistently increased ASAT activity, whereas all other laboratory results were within their respective reference ranges. Moreover, several studies have shown that macro-aspartate aminotransferase is the culprit in 40 to 100% of healthy cases with an isolated increase in ASAT activity.[4,6,10]

The presence of macroenzyme species can be determined by laboratory techniques including polyethylene glycol precipitation, size exclusion chromatography and protein electrophoresis. The polyethylene glycol precipitation technique is a low-cost method and can be used for the screening of all macroenzyme species (e.g. amylase, creatine kinase, prolactine). We recommend this method as a rapid initial screening method for the detection of macroenzyme species provided carefully defined protocols and reference ranges are used.[4,11]

Nowadays, several laboratory expert systems that permit real-time validation of biochemical data are readily available.[12,13] These automated systems use artificial intelligence techniques to aid decision making by the clinical chemist or physician. Clearly, a simple algorithm could trigger a reflex test strategy, including the polyethylene glycol precipitation technique and consequently prevent unwarranted investigations. Still, even in the absence of such expert systems, one should strongly consider the presence of a macroenzyme species if only a single enzyme activity is elevated.

Table 1. *Effect of polyethylene glycol (PEG) precipitation*

	ASAT activity (IU/l)		
	- PEG	+ PEG	%PPA
Patient	397	10	98
Control 1	210	160	24
Control 2	264	166	37

The polyethylene glycol-precipitable activity (% PPA) for ASAT was calculated as: % PPA = 100 x [(ASAT activity – ASAT activity PEG) / (ASAT activity)]; + PEG = 250 g PEG 6000 per litre 0.9% saline; - PEG = 0.9% saline.

CONCLUSION

Our case demonstrates the need to consider a macroenzyme species as a cause of persistent isolated elevation of ASAT to prevent unwarranted, invasive and expensive investigations. We recommend the use of the

polyethylene glycol precipitation technique as a simple and effective screening test for the detection of macroenzyme species.

REFERENCES

1. Remaley AT, Wilding P. Macroenzymes: Biochemical characterization, clinical significance and laboratory detection. Clin Chem. 1989; 35(12):2261-70.
2. Galasso PJ, Litin SC, O'Brien JF. The macroenzymes: a clinical review. Mayo Clin Proc. 1993;68:349-54.
3. Wilding P, Cooke WT, Nicholson GI. Globulin-bound amylase: a cause of persistently elevated levels in serum. Ann Intern Med. 1964;60:1053-9.
4. Davidson DF, Watson DJM. Macroenzyme detection by polyethylene glycol precipitation. Ann Clin Biochem. 2003;40:514-20.
5. Sabath LD, Gerstein DA, Finland M. Serum glutamic oxalacetic transaminase. False elevations during administration of erythromycin. N Engl J Med. 1968;279:1137-9.
6. Caropreso M, Fortunato G, Lenta S, et al. Prevalence and long-term course of macro-aspartate aminotransferase in children. J Pediatr. 2009;154(5):744-8.
7. Nagamine M, Okochi K. Complexes of immunoglobulins A and G with aspartate aminotransferase isoenzymes in serum. Clin Chem. 1983;29(2):379-81.
8. Wiltshire EJ, Crooke M, Grimwood K. Macro-AST: a benign cause of persistently elevated aspartate aminotransferase. J Paediatr Child Health. 2004;40(11):642-3.
9. Orlando R, Carbone A, Lirussi F. Macro-aspartate aminotransferase (macro-AST). A 12-year follow-up study in a young female. Eur J Gastroenterol Hepatol. 2003;15:1371-3.
10. Briani C, Zaninotto M, Forni M, Burra P. Macroenzymes: too often overlooked. J Hepatol. 2003;38(1):119.
11. Fahie-Wilson M, Halsall D. Polyethylene glycol precipitation: proceed with care. Ann Clin Biochem. 2008;45:233-5.
12. Valdiguié PM, Rogari E, Philippe H. VALAB: expert system for validation of biochemical data. Clin Chem. 1992;38(1):83-7.
13. Oosterhuis WP, Ulenkate HJ, Goldschmidt HM. Evaluation of LabRespond, a new automated validation system for clinical laboratory test results. Clin Chem. 2000;46(11):1811-7.

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PHOTO QUIZ

A case of abdominal tamponade

T.P. Saltzherr[1,3*], F. Lalezari[2], J.W. Gratama[2], P.E. Spronk[3]

Departments of [1]Surgery, [2]Radiology, [3]Intensive Care, Gelre Hospital, Apeldoorn, the Netherlands
*corresponding author: t.p.saltzherr@gelre.nl

CASE REPORT

A 44-year-old male was admitted with gastrointestinal bleeding. His medical history revealed alcohol addiction. Besides a haemoglobin level of 2.0 mmol/l, abnormal liver function tests were present. Recent history revealed melaena and haematemesis for several days. Gastroscopy showed a mild gastritis and gastroduodenal varices (grade 1-2). The patient was transfused, stabilised and admitted to the intensive care unit for further analysis and treatment. Ultrasonography of the abdomen was performed demonstrating a moderate amount of ascites and signs of liver cirrhosis including an enlarged liver with increased echogenicity with irregular appearing areas. Paracentesis and cultures did not reveal bacteria.

The next day the patient developed septic shock. Six out of six blood cultures showed *Morganella morganii* and ciprofloxacin was started. An abdominal computed tomography (CT) scan confirmed the suspected liver cirrhosis and ascites, although no abscess or clues for bowel perforation were seen. The patient's condition stabilised initially. On the 11th day the patient developed abdominal distension, hypotension requiring vasopressive medication, oliguria, and decreased pulmonary compliance. An abdominal CT scan was repeated (*figures 1* and *2*).

Figure 1. *Axial CT image showing intra-abdominal fluid and pneumoperitoneum. The large, white arrows indicate the cirrhotic liver with irregular superficial areas. The dotted arrows indicate the pneumoperitoneum*

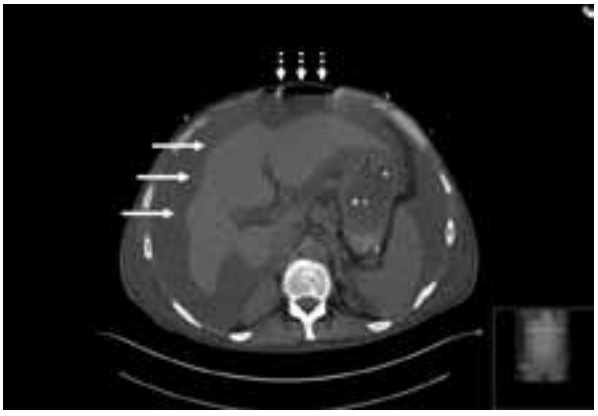
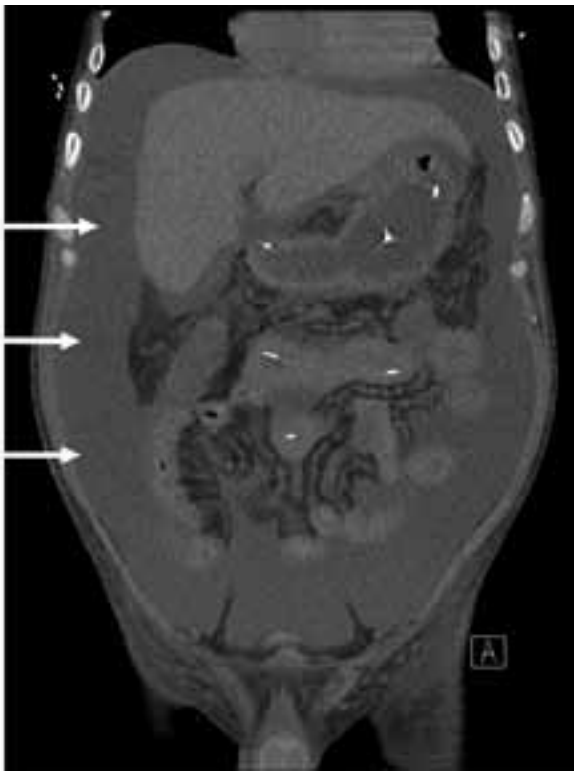


Figure 2. *Coronal CT image showing the massive fluid collection surrounding the intra-abdominal organs (white arrows)*



WHAT IS YOUR DIAGNOSIS ?

See page 142 for the answer to the photo quiz.

PHOTO QUIZ

Dripping candle wax

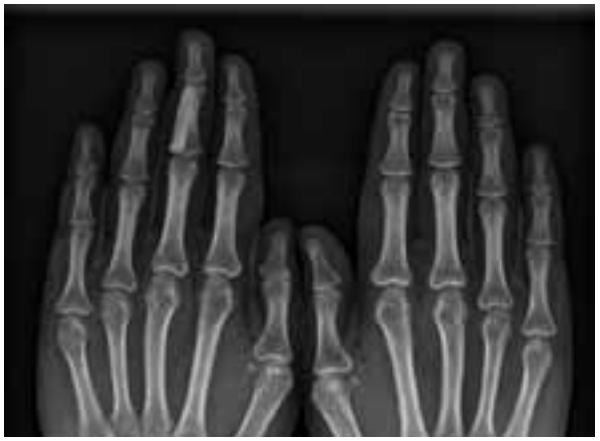
C.M.P.G. van Durme[1*], M.J.F. Starmans-Kool[2], H.R.M. Peeters[2]

*Corresponding author: e-mail: c.van.durme@mumc.nl

CASE REPORT

A 23-year-old woman presented with painful hands. There were no complaints of morning stiffness, night pain or swollen joints. Physical examination showed no signs of arthritis, no nail or skin lesions. Laboratory findings were normal: erythrocyte sedimentation rate 10 mm/h, C-reactive protein 8 mg/l, leucocytes 13.8 x 10^9/l, calcium 2.25 mmol/l, and alkaline phosphatase 104 U/l. The immune serology (antinuclear antibodies (ANA), anti-citrullinated protein antibodies (ACPA) and rheumatoid factor IgM) was negative. Plain radiography of the hands showed the following picture *(figure 1)*.

Figure 1. *The middle phalanx of the third finger of the left hand shows a typical picture of melorheostosis*



WHAT IS YOUR DIAGNOSIS ?

See page 143 for the answer to the photo quiz.

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PHOTO QUIZ

Life thru a lens

A. van Ratingen[1], M. Bollen[1], P.H. Bisschop[2], M.R. Soeters[2]*

[1]Department of Internal Medicine, IJsselmeerziekenhuizen, Lelystad, the Netherlands, [2]Department of Endocrinology and Metabolism, Academic Medical Center, Amsterdam, the Netherlands, *corresponding author: tel. +31 (0)20-5666071, fax: +31 (0)20-6917682, e-mail: m.r.soeters@amc.uva.nl

CASE REPORT

A 36-year-old man was referred with a four-month history of bone pain that was located in his left hip, lower back and ribs. At the same time he had difficulty in walking partly due to the pain and partly due to muscle weakness of his proximal extremities. His medical history mentioned congenital cataract due to aniridia, for which he received intraocular lens insertions. He was not on any medication. Physical examination showed a pale man wearing sunglasses. He had a prominent kyphoscoliosis. He reported pressure pain between the first lumbar and first sacral vertebrae and all movements of his extremities were painful. Further physical examination was normal. Neurological examination was also normal except for a slight paralysis of both ileopsoas muscles.

Magnetic resonance imaging of the spine showed multiple degenerative discs. X-ray of the hip joint was normal. Bone scintigraphy showed multiple osseous abnormalities, mainly in the ribs, left pubic bone and both sacroiliac (SI) joints (*figure 1*), which were suspicious for bone metastases of an unknown primary malignancy. Therefore, computer tomography (CT) scanning of the thorax and abdomen as well as laboratory tests were performed.

Figure 1. *Multiple osseous abnormalities, mainly in the ribs (left panel) and in the left pubic bone and both SI joints (right panel)*

CT imaging was completely normal, without obvious abnormalities in the vertebrae or other osseous structures. Laboratory tests showed a normal erythrocyte sedimentation rate (6 mm), slight leucocytosis (11 x 10^9/l), decreased calcium (2.08 mmol/l), normal albumin (37 g/l) decreased phosphate (0.58 mmol/l), and increased alkaline phosphatase (415 U/l).

WHAT IS YOUR DIAGNOSIS ?

See page 144 for the answer to the photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 139)

A CASE OF ABDOMINAL TAMPONADE

The diagnosis of abdominal compartment syndrome (ACS) was confirmed by measuring the intra-abdominal pressure (IAP), which proved to be 24 mmHg (normal value 5 to 7 mmHg). ACS occurs when the abdomen becomes subject to increased IAP which results in decreased perfusion of abdominal organs and impairs pulmonary function due to increased intrathoracic pressure. As dictated by the World Society of the Abdominal Compartment Syndrome,[1] the IAP should be indirectly measured via the bladder at end-expiration in a supine position.[2,3] This ensures that abdominal muscle contractions are absent. The transducer should be zeroed at the level of the mid-axillary line and instillation volume should not be more than 25 ml of saline. Measurements should be performed 60 seconds after installation to allow bladder detrusor muscle relaxation.

Several syndromes can cause ACS.[2] The treatment of ACS is aimed to reduce the IAP, e.g. improve the abdominal wall compliance, evacuate intra-abdominal fluid collections or remove intraluminal contents.[2] Furthermore, a definitive treatment for the primary cause of the raised IAP should be initiated.

In this case the subsequent diagnostic laparoscopy demonstrated a perforated caecum with faecal peritonitis as the cause of the ACS. Lavage of the peritoneum was carried out after drainage of six litres of infected ascites and a caecostomy was performed. After the intervention the intra-abdominal pressure dropped to 6 to 13 mmHg facilitating circulation and ventilation. Despite all measures taken, the patient died two days later.

Concluding, in patients with suspected abdominal compartment syndrome the intra-abdominal pressure should be measured and the underlying cause should be treated as soon as possible.

REFERENCES

1. WSACS; http://www.wsacs.org/
2. Cheatham ML, Malbrain ML, Kirkpatrick A, et al. Results from the International Conference of Experts on Intra-abdominal Hypertension and Abdominal Compartment Syndrome. II. Recommendations. Intensive Care Med. 2007;33(6):951-62.
3. De Keulenaer BL, De Waele JJ, Powell B, Malbrain ML. What is normal intra-abdominal pressure and how is it affected by positioning, body mass and positive end-expiratory pressure? Intensive Care Med. 2009;35(6):969-76.

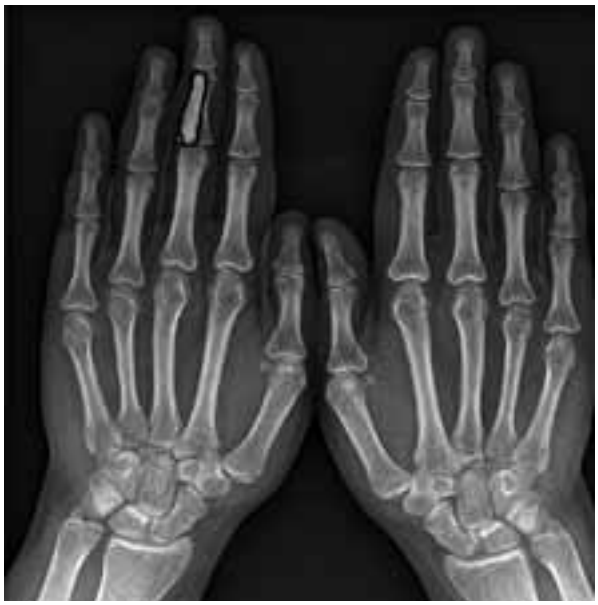
ANSWER TO PHOTO QUIZ (PAGE 140)

DRIPPING CANDLE WAX

The X-ray shows a very typical example of melorheostosis. The etymology of the word originates from the Greek 'melo' which means limb, ' rheos' which means flow and 'osteon' which means bone. It refers to the radiological aspect of a dripping candle wax. Melorheostosis is a rare disorder of bone development and belongs to the sclerotic bone dysplasia disorders. The cause is probably a loss-of-function mutation in LEMD3 gene, a protein involved in the development of intramembranous and endochondral bone.[1] It generally becomes manifest after early childhood, most cases being apparent by the age of 20 years. Mostly, it involves one limb but a single bone or multiple different bones may be affected. The symptoms vary from asymptomatic to pain, stiffness and limited range of motion. Most cases are benign, but a chronic course with progression of symptoms is also possible. The radiographic abnormalities of melorheostosis are sufficiently characteristic to allow accurate diagnosis in most cases.[2] Differential diagnosis comprises osteosarcoma in localised forms, myositis ossificans or calcified haematoma in cases associated with soft tissue calcifications. Treatment is generally symptomatic, but surgery might be needed in case of severe deformity or contractures.

It is not clear whether this coincidental finding in our patient is the cause of her pain. We treated her with painkillers (paracetamol) with good results.

Figure 1.



REFERENCES

1. Azouz EM, Greenspan A. Melorheostosis; Orphanet. http://www.orpha.net/data/patho/GB/uk-Melorheostosis.pdf.
2. Resnick D. Diagnosis of Bone and Joints Disorders. 3rd Edition. W.B. Saunders Company. Section XVIII, Muskuloskeletal disease. 1995;4410-4.

DIAGNOSIS

Altogether the laboratory tests and bone scan suggested a possible diagnosis of osteomalacia secondary to vitamin D deficiency, which was confirmed by an increased parathyroid hormone (84 pmol/l), and decreased 25(OH) vitamin D (12 nmol/l).

When asked specifically, the patient revealed that he suffered from severe photophobia due to the aniridia and had been avoiding daylight exposure for the past 20 years: he worked nightshifts, slept during the day and always kept his curtains closed. His food habits mentioned no dairy products or fish.

Osteomalacia is a disorder of decreased mineralisation of newly formed bone matrix.[1] Several different disorders cause osteomalacia, but vitamin D deficiency is the most common cause. Severe and prolonged vitamin D deficiency results in hypocalcaemia, secondary hyperparathyroidism, and hypophosphatemia, ultimately causing osteomalacia.[2] Moreover, vitamin D deficiency is associated with cardiovascular risk factors such as arterial hypertension, diabetes mellitus, chronic kidney disease and dyslipidaemia.[3]

Clinical manifestations of osteomalacia include diffuse bone pain, polyarthralgias, proximal muscle weakness and difficulty in walking.[1,4] The diagnosis is based on a combination of clinical manifestations, biochemical tests, radiological features such as pseudofractures (Looser zones) and, rarely, bone histomorphometry.[1,4]

Vitamin D deficiency should be corrected by supplementation of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) 50,000 IU (1.25 mg) once a week for eight weeks, followed by dose adjustments based on serum 25-hydroxyvitamin D and PTH levels.[1]

The extreme photophobia causing the vitamin D deficiency was due to congenital aniridia. Aniridia is a rare panocular disorder (incidence 1:64,000-1:100,000)[5,6] causing cataract, glaucoma and nystagmus and is due to mutations in the PAX6 gene on band p13 of chromosome 11.[5] Iris hypoplasia is the most obvious sign and leads to photophobia.

REFERENCES

1. Bhan A, Rao AD, Rao DS. Osteomalacia as a result of vitamin D deficiency. Endocrinol Metab Clin North Am. 2010;39(2):321-31.
2. Ashwell M, Stone EM, Stolte H, et al. UK Food Standards Agency Workshop Report: an investigation of the relative contributions of diet and sunlight to vitamin D status. Br J Nutr. 2010;104(4):603-11.
3. Pilz S, Tomaschitz A, März W, et al. Vitamin D, cardiovascular disease and mortality. Clin Endocrinol (Oxf). 2011;75(5):575-84.
4. Gifre L, Peris P, Monegal A, et al. Osteomalacia revisited: a report on 28 cases. Clin Rheumatol.2011;30(5):639-45.
5. Lee H, Khan R, O'Keefe M. Aniridia: current pathology and management. Acta Ophthalmol. 2008;86(7):708-15.
6. Adeoti CO, Afolabi AA, Ashaye AO, Adeoye AO. Bilateral sporadic aniridia: review of management. Clin Ophthalmol. 2010;4:1085-9.

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SPECIAL ARTICLE

Real-life costs of hepatitis C treatment

C.W. Helsper[1]*, H.L. Hellinga[1], G.A. van Essen[1], G.A. de Wit[1,2], M.J.M. Bonten[1], K.J. van Erpecum[3], A.I.M. Hoepelman[4], C. Richter[5], N.J. de Wit[1]

[1]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, the Netherlands, [2]Department of Prevention and Health Services Research, National Institute of Public Health and the Environment, Bilthoven, the Netherlands, Departments of [3]Gastroenterology, [4]Internal Medicine & Infectious Diseases, University Medical Center Utrecht, the Netherlands, [5]Department of Internal Medicine & Infectious Diseases, Rijnstate Hospital Arnhem, the Netherlands, corresponding author: tel.: +31 (0)6-14212460, fax: +31 (0)88-7568099, e-mail: C.W.Helsper-2@UMCUtrecht.nl

ABSTRACT

Background: Hepatitis C virus infection is a serious health threat in today's society. Improved identification strategies have increased the number of patients undergoing the expensive treatment with ribavirin and peg-interferon, inducing a substantial economic burden.

Methods: In a retrospective cohort study in three treatment centres in the Netherlands, files of patients treated between 2001 and 2010 were systematically searched for all cost-inducing treatment details. Costs of treatment resulting in sustained viral response (SVR), relapse, non-response and the costs per cured patient were specified for genotype and treatment setting. Determinants of costs were determined by multivariate linear regression.

Results: The mean 'real-life' treatment costs excluding side effects for genotype 1/4 and genotype 2/3 were approximately € 12,900 and € 9900 for all patients, € 15,500 and € 10,100 for treatment resulting in SVR and € 16,800 and € 12,100 for relapse, respectively. Costs per cured patient were € 28,500 and € 15,400 respectively. The costs of non-response were approximately € 8000 for all genotypes. Costs of side effects can be high and are mainly caused by incidental treatment for neutropenia. Medication is the main component of treatment costs. Treatment costs were higher in the academic setting due to longer duration and higher costs of side effects. Regression analysis confirms duration as the main determinant of treatment costs excluding side effects.

Conclusion: The 'real-life' costs of treatment are mainly determined by treatment duration, medication costs and costs of side effects. The costs of unsuccessful treatment are considerable as are the costs of side effects. Therefore, future research should aim at increasing SVR rates, reducing treatment duration and preventing side effects.

KEYWORDS

Costs, hepatitis C, treatment

INTRODUCTION

Due to its serious long-term complications, hepatitis C virus (HCV) infection is increasingly recognised as a serious health threat in today's society. An estimated 123 to 170 million people have been infected globally.[1,2] In the Netherlands, this number is estimated to be between 15,000 and 60,000.[3,4] An infection with HCV leads to chronic hepatitis in 80% of cases, of which 20% develop liver cirrhosis after 20 to 30 years. Of those with cirrhosis, approximately 5% develop hepatocellular cancer.[5] As a consequence of these severe long-term complications, HCV is considered responsible for 50 to 76% of all patients with liver cancer and two-thirds of all liver transplants in the Western world.[6]

The lack of clinical signs and low awareness among the general public and medical professionals have held back detection rates considerably, but in the past decade several successful identification strategies have been developed.[7,8] This has led to an increased number of patients eligible for and undergoing treatment, causing a substantial economic burden on society. Current success rates for treatment are dependent on genotype (GT). HCV infections have been found in seven genotypes of which genotype 1 to 4 are responsible for over 98% of the infections in the Netherlands.[9,10] Of the patients infected with genotype 1 and 4, approximately 50% can attain sustained viral response (SVR), which means the disease has been cured. The majority of patients infected with these genotypes require 48 weeks of treatment. For genotype 2 and 3 the treatment success rate is more favourable at approximately 80% after 24 weeks of treatment.[11]

Costs for HCV treatment result from several components. Direct medical costs result from professional workload, hospital costs, diagnostic testing, medication use and costs of side effects. Indirect costs result from societal burden, such as productivity losses associated with absence from work.

A national guideline for the treatment of chronic hepatitis C was developed in 2008, initiated by the Netherlands Association of Gastroenterologists and Hepatologists (Nederlandse Vereniging van Maag-Darm-Leverartsen).[12] This guideline provides recommendations for the initial evaluation, the choice of therapy and the required follow-up during and after therapy. This guideline aims to provide uniformity in treatment and a recent study has demonstrated that approximately 85% of treating medical specialists in gastroenterology, hepatology and internal medicine in the Netherlands adhere to the guidelines.[13]
The costs of treatment can diverge considerably as a result of varying treatment schedules and disease and patient characteristics. In this study we aim to assess the 'real-life' costs of successful HCV treatment, relapse after treatment, non-response and the costs per cured patient in the Netherlands. In addition, we aim to identify the most important determinants of these costs.

MATERIALS AND METHODS

A retrospective cohort study was performed in three main HCV treatment centres in the Netherlands (two academic, one non-academic). The files of patients treated for HCV between 2001 and 2010 were systematically searched for details of treatment. The cooperating treatment centres provided the files of eligible patients, according to the following exclusion criteria: treatment other than ribavirin and peg-interferon, previous HCV treatment, HIV co-infection, unclosed files (meaning not designated as fully completed by the treating physician), excessive missing data (e.g. due to change of treatment institution) and no information available on treatment outcome. The researchers checked the provided files for eligibility in the study. In close cooperation with the treating physicians, the data were extracted anonymously from the electronic and paper patient files. In these files all cost-inducing elements were systematically extracted. These include the number of consultations, admissions to the hospital and length of stay, medication use, number and type of diagnostic tests performed, use of specialised homecare (e.g. 'Pegassist' or 'HepaZorg') and other registered use of hospital facilities. Side effects were recorded based on the available reporting in patient files and additional diagnostic testing or treatment outside of the protocol related to side effects known for HCV treatment. All data from one month before the beginning of drug treatment until the evaluation of treatment success at 24 weeks after drug treatment had ended were included in the analyses. Diagnostic testing as recommended in the national protocol and performed less than one year previous to the beginning of drug treatment was also taken into account. The costs resulting from the aforementioned treatment aspects were retrieved from the financial departments of the treating centres and the Dutch Health Care Insurance Board.[14] The latter costs are standardised cost prices that are recommended for use in health economic evaluations. Indirect costs, such as absence at work due to sickness, were not included in the calculations. Hence, the current study takes a healthcare perspective and estimates costs for 2010.
Mean treatment costs were determined for the different treatment outcomes and linked to the available patient and treatment characteristics. In addition, the 'costs per cure' were calculated by dividing the sum of treatment costs of all patients by the number of patients attaining SVR. The latter provides an indication of the average investment required for curing disease in one patient. Patient and treatment characteristics include age, gender, relevant lifestyle such as known hard drug use, presence of co-infections such as HBV, genotype, liver damage based on the Metavir classification (determined by biopsy or fibroscan), treatment duration and treatment setting.[15] In addition, the theoretical costs resulting from a full term and strictly followed treatment schedule according to the national treatment protocol were calculated as background information.
To detect the most important patient and treatment characteristics determining treatment costs, we performed multivariate linear regression. This analysis was performed in two steps, the first excluding and the second including 'severity of liver damage' as a parameter in the model. The first analysis was performed for all 85 treated patients and repeated for both groups of genotypes. Since information on severity of liver damage could only be found in the files of 59 patients (40 with GT1/4 and 19 with GT2/3), the impact of this parameter on treatment costs was determined in a separate analysis.

RESULTS

From the study period, 104 patient files were provided by the three treatment centres, which were considered to match the inclusion criteria, out of an estimated 150 to 200 patients treated with peg-interferon and ribavirin. After strict application of the exclusion criteria by the research team, the files of 85 patients proved suitable for analyses. The main reasons for secondary exclusion by the researchers were a positive HIV status, unclosed files or change of treating institution. Baseline characteristics are demonstrated in

table 1. The mean costs and duration of HCV treatment and the corresponding standard deviations (SD), specified for patients with GT1/4 and GT2/3 and for treatment result, are shown in *table 2*. This table also includes costs per cure. *Figure 1* demonstrates the different treatment costs for different outcomes, specified for costs of diagnostic testing, medication, hospital costs and side effects.

We found a substantial variability in costs of side effects, which was caused by only a few patients with very high costs and therefore largely determined by chance. Therefore, the primary presentation of costs is done excluding side effects with the costs of side effects presented separately.

Costs of treatment for all patients

The mean costs of treatment for all treated patients with GT1/4, excluding costs of side effects, were approximately € 12,900 after a mean treatment duration of 223 days (31.8 weeks). Mean costs of side effects were approximately € 2200. The nature of the side effects responsible for these costs is provided in the paragraphs below.

The mean treatment costs for all patients with GT2/3, excluding costs of side effects, were approximately € 9900 after a mean treatment duration of 174 days (24.8 weeks). Mean costs of side effects were approximately € 2400.

The theoretical costs of a full-term treatment based on the national protocol were € 19,189 for GT1/4 and € 11,204 for GT2/3.

Costs of successful treatment

SVR was attained in 53% of patients with GT1/4 and 74% of patients with GT2/3.

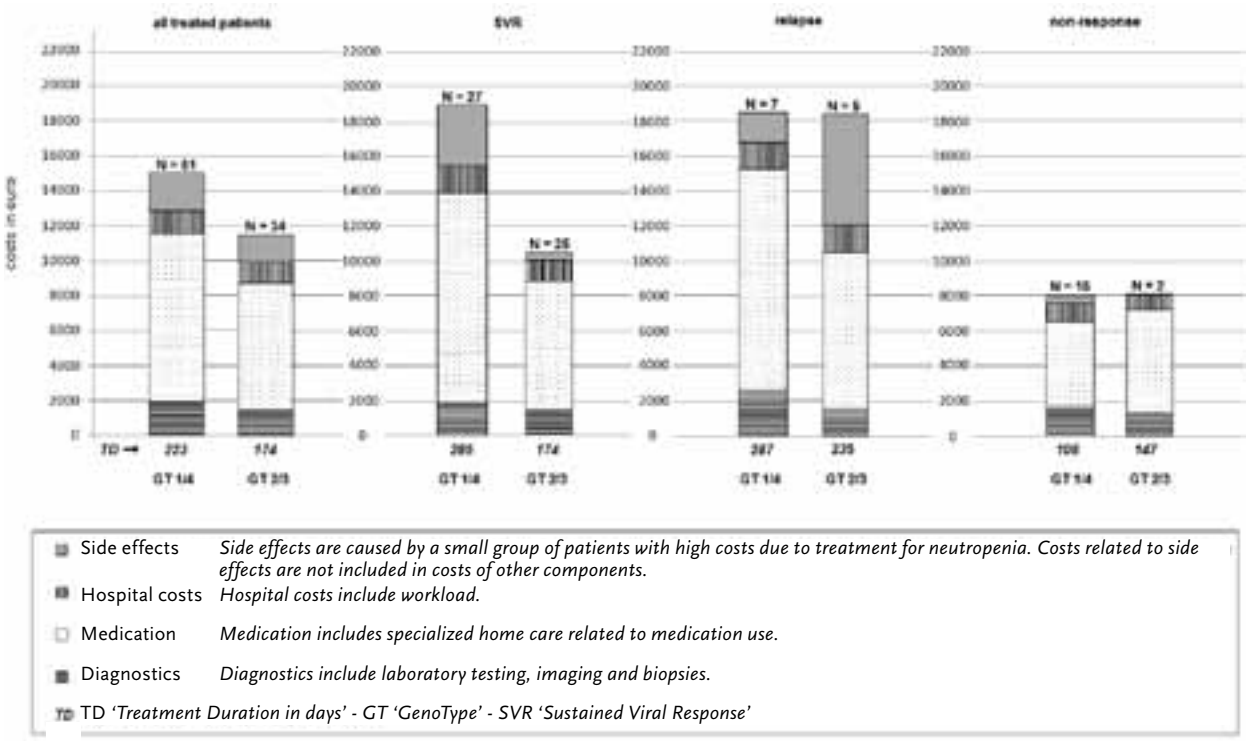
The mean costs of treatment resulting in SVR for patients with GT1/4, excluding costs of side effects, were approximately € 15,500 after a mean treatment duration of 285 days (40.7 weeks). Mean costs of side effects were approximately € 3500. The considerable costs of side effects were generated by six patients in the academic setting for whom € 5818 to € 41,543 was spent on the treatment of side effects. These high costs result from treatment with pegfilgrastim for neutropenia and epoetin alfa for anaemia.

Table 1. *Patient characteristics in different settings*

	All settings	Academic Department of Infectious Diseases	Academic Department of Gastroenterology	Non-academic Department of Infectious Diseases
Genotype 1 and 4				
Number of patients	51	15	14	22
Gender – male	40 (78%)	12 (80%)	10 (71%)	18 (82%)
Mean age	46.4	46.1	44.1	48.0
Liver damage known†	40 (78%)	14 (93%)	13 (93%)	13 (59%)
- No scarring	12 (30%)	4 (29%)	4 (31%)	4 (31%)
- Minimal scarring	7 (18%)	2 (14%)	2 (15%)	3 (23%)
- Moderate scarring	9 (23%)	3 (21%)	3 (23%)	3 (23%)
- Bridging fibrosis	6 (15%)	5 (36%)	1 (8%)	0 (0%)
- Cirrhosis or advanced scarring	6 (15%)	0 (0%)	3 (23%)	3 (23%)
Sustained viral response	27 (53%)	9 (60%)	7 (50%)	11 (50%)
Mean treatment duration in days (SD)	223 (120)	260 (144)	225 (103)	196 (110)
Genotype 2 and 3				
Number of patients	34	13	7	14
Gender – male	26 (76%)	9 (69%)	6 (86%)	11 (79%)
Mean age	42.5	39.8	48.4	42.1
Liver damage known †	19 (56%)	9 (69%)	7 (100%)	3 (21%)
- No scarring	5 (26%)	5 (56%)	0 (0%)	0 (0%)
- Minimal scarring	5 (26%)	3 (33%)	2 (29%)	0 (0%)
- Moderate scarring	4 (21%)	1 (11%)	2 (29%)	1 (33%)
- Bridging fibrosis	1 (5%)	0 (0%)	1 (14%)	0 (0%)
- Cirrhosis or advanced scarring	4 (21%)	0 (0%)	2 (29%)	2 (67%)
Sustained viral response	25 (73%)	11 (85%)	3 (43%)*	11 (79%)
Mean treatment duration in days (SD)	174 (70)	190 (69)	156 (99)*	167 (56)

***Low number due to two dropouts with early side effects; †Based on Metavir classification for liver damage; 1. no scarring, 2. minimal scarring, 3. scarring has occurred and extends outside the areas in the liver that contain blood vessels, 4. bridging fibrosis is spreading and connecting to other areas that contain fibrosis, 5. cirrhosis or advanced scarring of the liver.[15]**

Figure 1. *Components of costs of treatment, specified for treatment outcome**



***Four patients, two with GT1/4 and two with GT2/3, stopped treatment due to side effects after a mean duration of 31.5 days and total treatment costs of 3.796 euro. These patients are only included in the 'All patients' group.**

Table 2. *Costs (€) and duration (days) of treatment – specified for treatment result*

	All patients † Mean *SD*	***SVR*** † Mean *SD*	***Relapse*** † Mean *SD*	***Non-response*** † Mean *SD*	***Costs per cure*** ‡
Genotype 1 and 4	*n=51**	*n=27*	*n=7*	*n=15*	
Costs excluding side effects	12,856	15,483	16,800	7566	24,283
	6060	*4980*	*5466*	*2840*	
Costs including side effects	15,104	19,032	18,464	8,014	28,529
	9010	*9293*	*5502*	*2833*	
Mean treatment duration in days	223	285	287	108	
	120	*90*	*94*	*53*	
Costs if national protocol completed	19,189				
Genotype 2 and 3	*n=34*	*n=25*	*n=5*	*n=2*	
Costs excluding side effects	9911	10,095	12,068	8065	13,479
	3051	*2574*	*3490*	*184*	
Costs including side effects	11,324	10,757	18,340	8078	15,400
	7175	*3392*	*16,151*	*202*	
Mean treatment duration in days	174	174	235	147	
	70	*55*	*92*	*30*	
Costs if national protocol completed	11,204				

†Mean treatment costs and mean treatment duration of patients with the indicated outcome. ‡Costs per cure were calculated by dividing the sum of treatment costs of all patients by the number of patients attaining SVR. This provides an indication of the investment made for curing disease in one patient. *Four patients, two with GT1/4 and two with GT2/3, stopped treatment due to side effects after a mean duration of 31.5 days and mean treatment costs of € 3796. These patients are only included in the 'All patients' group.

The mean costs of treatment resulting in SVR for those with GT2/3, excluding costs of side effects, were approximately € 10,100 after a mean treatment duration of 174 days (24.8 weeks). Mean costs of side effects were lower at approximately € 650.

Costs per cure for patients with GT1/4 were approximately € 28,500 including side effects and € 24,300 excluding side effects. Costs per cure for patient with GT2/3 were approximately € 15,400 including side effects and € 13,500 excluding side effects.

Costs of unsuccessful treatment

The mean costs of treatment of patients with GT1/4 resulting in relapse after initial success, excluding side effects, were € 16,800 after a treatment duration of 287 days (41.0 weeks). Mean costs of side effects were approximately € 1700. The mean costs of treatment for patients with GT1/4 resulting in non-response, excluding side effects, were € 7600 after a treatment duration of 108 days (15.4 weeks). Mean costs of side effects were approximately € 450.

The mean costs of treatment of patients with GT2/3 resulting in relapse after initial success, excluding side effects, were € 12,100 after a treatment duration of 235 days (33.6 weeks). Mean costs of side effects were approximately € 6300. These costs of side effects were high due to the treatment of one patient in the academic setting, who received filgrastim for neutropenia costing approximately € 31,000. The mean costs of treatment for patients with GT2/3 resulting in non-response, excluding side effects, were € 8100 after a treatment duration of 147 days (21.0 weeks). Mean registered costs of side effects were only € 13 in this group.

Determinants of treatment costs

As demonstrated by the aforementioned findings, costs of side effects were substantial and consequently an important component of total treatment costs. As demonstrated in *figure 1*, the primary constituent of the treatment costs was the cost of medication.

The multivariate linear regression analyses indicated that treatment duration was the sole statistically significant determinant of treatment costs in all separate analyses (p value <0.001). Genotype, gender, age, known injecting drug use, treatment setting, somatic comorbidity, psychiatric comorbidity and severity of liver damage were not independently associated with treatment costs (p value >0.05). *Tables 3* and *4* demonstrate the full results of the multivariate analyses.

Treatment setting

Table 5 provides an overview of the mean treatment costs specified for treatment setting and outcome.

Table 3. *Multivariate linear regression – determinants of treatment costs excluding side effects in a model excluding liver damage*

	Unstandardised coefficients (B)	Standardised coefficients (Beta)	P value
All patients - (n=85)			
Genotype 2	-809.6	-0.043	0.231
Genotype 3	-442.0	-0.039	0.315
Genotype 4	-11.1	-0.001	0.985
Treatment result	51.6	0.009	0.836
Gender (1=male)	289.6	0.023	0.519
Age	18.0	0.039	0.272
Treatment duration (days)	*47.4*	*0.950*	*0.000*
Known injecting drug use (1=yes)	-301.2	-0.029	0.436
Treatment setting (1=non-academic)	-494.3	-0.047	0.171
Somatic comorbidity	-556.2	-0.037	0.295
Psychiatric comorbidity	-244.5	-0.022	0.524
(Constant)	*1660.8*		*0.149*
(Explained variance – R^2)	*0.927*		
Genotype 1 and 4 - (n=51)			
Treatment result	662.7	0.108	0.082
Gender (1=male)	657.7	0.045	0.267
Age	10.7	0.022	0.596
Treatment duration (days)	*53.0*	*1.048*	*0.000*
Known injecting drug use (1=yes)	-291.5	-0.024	0.547
Treatment setting (1=non-academic)	-446.9	-0.037	0.345
Somatic comorbidity	-794.9	-0.048	0.243
Psychiatric comorbidity	-213.4	-0.017	0.665
(Constant)	*-646.9*		*0.663*
(Explained variance – R^2)	*0.946*		
Genotype 2 and 3 - (n=34)			
Treatment result	-603.3	-0.170	0.060
Gender (1=male)	504.4	0.071	0.407
Age	-2.1	-0.006	0.935
Treatment duration (days)	*36.5*	*0.838*	*0.000*
Known injecting drug use (1=yes)	68.2	0.011	0.902
Treatment setting (1=non-academic)	-419.6	-0.069	0.369
Somatic comorbidity	829.2	0.089	0.291
Psychiatric comorbidity	-763.6	-0.112	0.151
(Constant)	*4389.1*		*0.005*
(Explained variance – R^2)	*0.870*		

In the academic setting costs per cure were 33% higher for GT1/4 and 43% higher for GT2/3, than in the non-academic setting. After adjustment for costs of side effects, this difference remained at 14 and 21%. For GT2/3, this is mainly the result of the low SVR rate in one of the academic centres in which only 43% (3 out of 7) patients reached SVR. This low SVR rate was caused by two early drop-outs due to side effects and two patients who relapsed.

Table 4. *Multivariate linear regression – determinants of treatment costs excluding side effects in a model including liver damage*

	Unstandardised coefficients (B)	Standardised coefficients (Beta)	P value
All patients - (n=59)			
Genotype 2	-1319.7	-0.059	0.181
Genotype 3	-206.6	-0.016	0.757
Genotype 4	-77.3	-0.004	0.921
Treatment result	257.1	0.045	0.472
Gender (1=male)	416.5	0.033	0.483
Age	14.8	0.031	0.512
Treatment duration (days)	*49.2*	*0.986*	*0.000*
Known injecting drug use (1=yes)	-264.1	-0.023	0.602
Treatment setting (1=non-academic)	-856.3	-0.067	0.113
Somatic comorbidity	-659.5	-0.042	0.379
Psychiatric comorbidity	-190.5	-0.016	0.729
Severity of liver damage	-107.2	-0.027	0.597
(Constant)	*1359.8*		*0.376*
(Explained variance – R^2)	*0.934*		
Genotype 1 and 4 - (n= 40)			
Treatment result	845.6	0.132	0.150
Gender (1=male)	845.6	0.058	0.305
Age	9.3	0.019	0.732
Treatment duration (days)	*54.4*	*1.076*	*0.000*
Known injecting drug use (1=yes)	-358.9	-0.028	0.577
Treatment setting (1=non-academic)	-574.8	-0.043	0.370
Somatic comorbidity	-689.6	-0.039	0.524
Psychiatric comorbidity	-380.3	-0.029	0.593
Severity of liver damage	-114.9	-0.026	0.667
(Constant)	*-999.9*		*0.644*
(Explained variance – R^2)	*0.940*		
Genotype 2 and 3 - (n=19)			
Treatment result	-565.1	-0.180	0.101
Gender (1=male)	-23.4	-0.004	0.976
Age	-36.6	-0.102	0.297
Treatment duration (days)	*32.0*	*0.769*	*0.000*
Known injecting drug use (1=yes)	387.9	0.065	0.614
Treatment setting (1=non-academic)	-1091.8	-0.135	0.207
Somatic comorbidity	-291.1	-0.036	0.757
Psychiatric comorbidity	-1234.3	-0.171	0.156
Severity of liver damage	464.4	0.229	0.151
(Constant)	*5989.0*		*0.004*
(Explained variance – R^2)	*0.947*		

An additional explanation for the higher costs in the academic setting is the longer mean treatment duration. For GT1/4, the number of 'treatment days per cure' in the academic setting is 441 days *vs* 392 in the non-academic setting (difference 12%). For GT2/3 this difference is 255 *vs* 213 days (difference 20%). The highest and lowest mean number of treatment days correspond with the highest and lowest SVR rates.

The mean total costs of treatment for all patients were approximately 50% higher in the academic setting at € 17,500 *vs* € 12,000 in the non-academic setting. Adjustment for investments made for the treatment of side effects reduces this difference to approximately 25% (€ 14,100 *vs* € 11,100). This resembles the difference in treatment duration, which is also approximately 25% (243 *vs* 196). Consequently the treatment costs per day were similar at € 58.1 in the academic setting *vs* € 57.2 in the non-academic setting. For treatment leading to SVR these costs were € 54 (academic) and € 55 (non-academic).

DISCUSSION

Summary of findings

The mean 'real-life' costs for all patients treated for HCV – excluding side effects – were € 12,900 for GT 1/4 and € 9900 for GT 2/3, while costs were slightly higher for treatments resulting in SVR. Treatment resulting in relapse increased costs by approximately € 2000. Non-response costs approximately € 8000 for all genotypes. Costs per cured patient including side effects are approximately € 28,500 and € 15,400 for GT1/4 and GT2/3. Costs of side effects can be substantial and differ considerably between patients. Treatment duration and medication costs were the most important determinants of total costs. Treatment costs were generally higher in the academic setting.

The finding that the higher costs in the academic setting result from longer duration is supported by the multivariate regression analyses which demonstrate that, when corrected for treatment duration, treatment setting is not associated with costs of treatment.

The higher costs of treatment for side effects in the academic setting can in part be explained by the treatment used for anaemia. In the academic setting epoetin alfa was used in the treatment of anaemia, whereas in the non-academic setting the treatment was based on blood transfusions. The main difference in costs of side effects, however, results from the treatment of neutropenia with (peg)filgrastim. Since this treatment should only be initiated and supervised by physicians with relevant experience, availability of this specialised care in the different settings at the time of treatment could have been of influence. The readiness and possibilities to invest more in attaining SVR could be another reason for the difference in costs of side effects. However, the costs of side effects and corresponding success rates of the different settings do not reflect this. Since the number of patients generating the costs of side effects is low, the difference in costs of side effects between settings could also be due to chance. The

Table 5. *Mean treatment costs (€) and duration – specified for treatment setting and outcome*

	All patients	SVR	Relapse	Non-response	Early stop	Costs per cure
Genotype 1 and 4	***n = 51***	***n = 27***	***n = 7***	***n = 15***	***n = 2***	
Costs excluding side effects						
All patients	12,856	15,483	16,800	7566	3266	24,283
Non-academic	11,199	13,856	15,320	6000	-	22,398
Academic	14,113	16,601	17,911	9355	3266	25,580
- Gastroenterology	13,351	15,322	15,898	11,094	3486	26,702
- Infectious diseases	14,824	17,596	19,924	7036	3045	24,707
Costs including side effects						
All patients	15,104	19,032	18,464	8014	3483	28,529
Non-academic	11,929	14,535	16,985	6449	-	23,858
Academic	17,512	22,124	19,573	9802	3483	31,741
- Gastroenterology	18,320	24,789	17,331	11,094	3921	36,640 †
- Infectious diseases	16,758	20,051	21,815	8079	3045	27,930
Mean treatment duration						
All patients	223	285	287	108	21	-
Non-academic	196	250	296	84	-	-
Academic	243	309	280	135	21	-
- Gastroenterology	225	285	224	172	21	-
- Infectious diseases	260	327	336	86	21	-
Genotype 2 and 3	***n = 34***	***n = 25***	***n = 5***	***n = 2***	***n = 2***	
Costs excluding side effects						
All patients	9911	10,095	12,068	8065	4061	13,479
Non-academic	9480	9154	12,045	7935	-	12,066
Academic	10,212	10,834	12,083	8195	4061	14,589
- Gastroenterology	9701	11,125	13,203	-	4061	22,635 *
- Infect ious diseases	10,488	10,754	9843	8195	-	12,394
Costs including side effects						
All patients	11,324	10,757	18,340	8,078	4110	15,400
Non-academic	9755	9503	12,050	7935	-	12,415
Academic	12,422	11,742	22,534	8221	4110	17,745
- Gastroenterology	15,109	13,261	28,879	-	4110	35,253 *
- Infectious diseases	10,975	11,328	9843	8221		12,971
Mean treatment duration						
All patients	174	174	235	147	42	-
Non-academic	167	155	252	126	-	-
Academic	178	189	224	168	42	-
- Gastroenterology	156	168	252	-	42	-
- Infectious diseases	190	194	168	168	-	-

*** High costs due to low sustained viral response rate (43% of total n=7, including 2 early dropouts due to side effects); † High costs due to large investment in treatment of a few patients for side effects.**

readiness and possibilities to invest more in realising SVR also provides an explanation for the difference in treatment duration between the treatment settings. This is supported by the finding that longer treatment duration seemed to be related to increased success rate. Since our study was neither designed nor aimed to assess the determinants of treatment success, we did not test this relationship in detail.

A final explanation for the longer treatment duration and higher costs for side effects in the academic setting are differences in baseline characteristics of the patient populations. In general, primary care physicians choose to send patients in whom a more complicated treatment is expected to more specialised settings such as the academic centres. In addition, less specialised medical specialists sometimes refer HCV patients who are difficult to treat to more specialised treatment centres. Due to the limited number of patients for whom liver damage could be determined and the lack of information on patient characteristics which could influence treatment success (such as BMI or ethnicity), we could not test this hypothesis.

Strengths and limitations

The main strength of our study is that it provides a 'real-life' overview of the costs of treatment as performed in daily practice instead of a theoretical profile prescribed by the protocol. This leads to a daily practice-based estimation of treatment costs assessed in a 'real-life' population. Given the highly variable population treated for HCV and the various factors which could lead to treatment adjustment, we expect that our 'real-life' study provides a more reliable estimation of treatment costs than theory-based estimations.

The main limitation is that the input for our calculations is restricted to the data that are registered in the patient files. This might have led to an underestimation of true costs, due to omissions. This underestimation is likely to be most relevant for side effects, because files will only state what is substantial or complained about. Registrations of side effects based on diagnostic testing outside the protocol will only detect side effects for which additional diagnostic testing is needed. In addition, only side effects for which it was certain that they were caused by the HCV treatment were registered as being a side effect. Comorbidities existing previous to treatment and flaring up during treatment were not included because treatment could not be confirmed as the causal factor. This might lead to an underestimation of side effects and their costs. Diagnostic testing is automatically reported by the hospital systems and costs of medication were calculated based on the initiated treatment and reported changes in medication dosage. Therefore underestimation of costs for these determinants is expected to be minor.

Given the retrospective and therefore observational data collection, we had limited influence on registration of patient characteristics which are related to treatment outcome. Consequently we had incomplete knowledge of characteristics which could have provided more background on the reasons for longer treatment duration and higher costs of treatment, such as liver damage, specified psychiatric problems, BMI, race and alcohol dependency.

The fact that our data came from three treatment centres may limit the generalisability of the conclusions. However, HCV treatment is concentrated in a limited number of expertise centres and the three centres in our study cover most of the HCV treatment in the central and eastern region of the Netherlands.

Even though the costs of HCV treatment are substantial, the healthcare costs of not treating HCV are estimated to be much higher due to development of complications such as cirrhosis and hepatocellular cancer if HCV is left untreated.[16] Consequently, as demonstrated by previous studies, timely identification and treatment with HCV is likely to be cost-effective.[7,17] In addition, recent findings demonstrate increased treatment success rates, particularly for genotype 1, Even though this will initially result in higher treatment costs, it will eventually lead to further improvement of cost-effectiveness.[18-20]

As a result of the exclusion criteria, our cohort does not cover the full range of HCV patients. Consequently, our results are best applicable for HIV-negative patients undergoing primary HCV treatment. This might have led to an underestimation of the overall costs, since costs for the excluded population are expected to be somewhat higher.

CONCLUSION

The 'real-life' costs of HCV treatment are mainly determined by the costs of medication and side effects and the duration of treatment. At present, these determinants and the costs of treatment for patients who are not treated successfully lead to a substantial financial investment needed to cure one patient. To reduce costs and improve cost-effectiveness of treatment, future research should be aimed at increasing SVR rates, reducing treatment duration and preventing side effects.

ACKNOWLEDGEMENTS

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There are no conflicts of interest to be reported.

REFERENCES

1. Hepatitis C: global prevalence. Wkly Epidemiol Rec 1997;72:341-4.
2. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis. 2005;5:558-67.
3. Health Council of the Netherlands: Committee on hepatitis C. Detection and treatment of people with hepatitis C. Rijswijk; 1997. Report No.: 1997/19.
4. Slavenburg S, Verduyn-Lunel FM, Hermsen JT, et al. Prevalence of hepatitis C in the general population in the Netherlands. Neth J Med. 2008;66:13-7.
5. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med. 2001;345:41-52.
6. World Health Organization (WHO). State of the art of vaccine research and development. Report number WHO/IVB/05.XX. Available from http://www.who.int/vaccine_research/documents/Viral_Cancers.pdf; 2011.
7. Helsper CW, Borkent-Raven BA, de Wit NJ, et al. Cost-effectiveness of targeted screening for hepatitis C in the Netherlands. Epidemiol Infect. 2011;16:1-12.
8. Zuure FR, Davidovich U, Coutinho RA, et al. Using mass media and the Internet as tools to diagnose hepatitis C infections in the general population. Am J Prev Med. 2011;40:345-52.

9. de Vries MJ, te Rijdt B, van Nieuwkerk CM. Transmission of hepatitis C genotypes in the Netherlands amongst recently genotyped patients. Neth J Med. 2008;66:40-1.

10. de Vries MJ, te Rijdt B, van Nieuwkerk CM. Genotype distribution amongst hepatitis C patients in the Netherlands. Neth J Med. 2006;64:109-13.

11. Manns MP, Foster GR, Rockstroh JK, et al. The way forward in HCV treatment--finding the right path. Nat Rev Drug Discov. 2007;6:991-1000.

12. de Bruijne J, Buster EH, Gelderblom HC, et al. Treatment of chronic hepatitis C virus infection – Dutch national guidelines. Neth J Med. 2008;66:311-22.

13. Slavenburg S, Lamers MH, Roomer R, et al. Current clinical care compared with new Dutch guidelines for hepatitis C treatment. Neth J Med. 2009;67:177-81.

14. Oostenbrink JB, Bouwmans C.A.M., Koopmanschap MA, Rutten FF, Jager J, institute for medical technology assessment EM. Standardisation of costs: the Dutch Manual for Costing in economic evaluations. [in Dutch; Handleiding voor kostenonderzoek: methoden en standaard kostprijzen voor economische evaluaties in de gezondheidszorg] - Update 2004.

15. Theise ND. Liver biopsy assessment in chronic viral hepatitis: a personal, practical approach. Mod Pathol. 2007;20 Suppl 1:S3-14.

16. Leigh JP, Bowlus CL, Leistikow BN, Schenker M. Costs of hepatitis C. Arch Intern Med. 2001 Oct 8;161:2231-7.

17. Sroczynski G, Esteban E, Conrads-Frank A, et al. Long-term effectiveness and cost-effectiveness of antiviral treatment in hepatitis C. J Viral Hepat. 2010 Jan;17:34-50.

18. Poordad F, McCone J, Bacon BR, et al. Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med. 2011;364:1195-206.

19. Bacon BR, Gordon SC, Lawitz E, et al. Boceprevir for previously treated chronic HCV genotype 1 infection. N Engl J Med. 2011;364:1207-17.

20. Gevers TJ, Slavenburg S, van Oijen MG, Drenth JP. Treatment extension benefits HCV genotype 1 patients without rapid virological response: a systematic review. Neth J Med. 2011;69:216-21.

LETTER TO THE EDITOR

Lyme disease – the challenge for patients

S.R. Huyshe-Shires, S. Pearson

Lyme Disease Action, PO Box 235, Penryn, Cornwall, TR10 8WZ, UK

Dear Editor,

'The challenge of Lyme disease: tired of Lyme wars' by Kullberg *et al.*[1] came as a welcome relief for patients with Lyme disease. The dilemma of the treating physician and patient confronted with a limited evidence base is the central theme. The uncertainties which threaten to undermine the doctor-patient relationship are acknowledged with the emphasis on further research, proper care and resolution rather than conflict.

In contrast, the editorial 'Lyme borreliosis: the challenge of accuracy' by Klempner *et al.*[2] portrays the Kullberg editorial as a 'plea' and the language of conflict is resurrected with references to 'the field' and to 'standards' with patient support groups redefined as 'activists'.

The Klempner editorial attempts to defend the Klempner trials[3] against the criticism of Kullberg, stating 'Klempner *et al.* did not find any evidence, based on over 700 samples from 129 patients that were examined by culture and polymerase chain reaction (PCR) assays, for persistence of *B. burgdorferi* sensu stricto infection in patients with persistent treatment for Lyme borreliosis.'

Despite low sensitivity, an exclusion criterion for the original study was a positive PCR result for *B. burgdorferi* DNA in plasma or cerebrospinal fluid. It is predictable that 129 baseline blood samples and 128 cerebrospinal fluid samples tested negative for *B. burgdorferi* DNA and that 458 blood samples during treatment continued to test negative, giving a total of 715 negative PCR results. This degree of selection bias with absent data on blood cultures cannot be accepted as 'lack of evidence of persistence'. Eight patients (6.25%) did show evidence of intrathecal antibody production.

Since the Klempner editorial was published, a further European study has corroborated Kullberg's view that the performance of serological assays is suboptimal.[4]

Patients are disheartened by doctors apparently trying to score points off one another instead of directing their expertise towards resolving undeniable uncertainties. The challenge for patients struggling with Lyme disease is not academic. Uncertainty, fear, pain and hardship can seem endless. How do patients understand that although the Infectious Diseases Society of America (IDSA) recommends further treatment for continuing objective arthritis, subjective pain does not count? Or that because fatigue is non-specific a trial demonstrating significant improvement[5] is considered a failure?

Lack of good quality evidence concerning diagnosis and treatment disempowers doctors and all too often disenfranchises and alienates patients. Unfortunately the recent editorial by Klempner *et al.*, by foreclosing valid questions, can only perpetuate this state of affairs.

REFERENCES

1. Kullberg BJ, Berende A, van der Meer JWM. The challenge of Lyme disease: tired of Lyme wars. Neth J Med. 2011;69:98-100.
2. Klempner MS, Halperin JJ, Baker PJ, et al. Lyme borreliosis: the challenge of accuracy. Neth J Med. 2012;70:3-5.
3. Klempner MS, Hu LT, Evans J, et al. Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. N Engl J Med. 2001;345:85-92.
4. Müller I, Freitag MH, Poggensee G, et al. Evaluating Frequency, Diagnostic Quality, and Cost of Lyme Borreliosis Testing in Germany: A Retrospective Model Analysis. Clin Dev Immunol. 2012;2012:595427.
5. Krupp LB, Hyman LG, Grimson R, et al. Study and treatment of post Lyme disease (STOP-LD): A randomized double masked clinical trial. Neurology. 2003;60(12):1923-30.

Netherlands
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Valproic acid-induced DRESS syndrome with acute liver failure

M.A.D. van Zoelen[1]*, M. de Graaf[2], M.R. van Dijk[3], A. Bogte[4], K.J. van Erpecum[4], H. Rockmann[2], L.J. Maarschalk-Ellerbroek[1]

Departments of [1]Internal Medicine, [2]Dermatology, [3]Pathology and [4]Gastroenterology; University Medical Center Utrecht (UMCU), Utrecht, the Netherlands, *corresponding author: tel: +31 (0)88-7555555, fax: +31(0)30-2518328, e-mail: M.A.D.vanZoelen@umcutrecht.nl

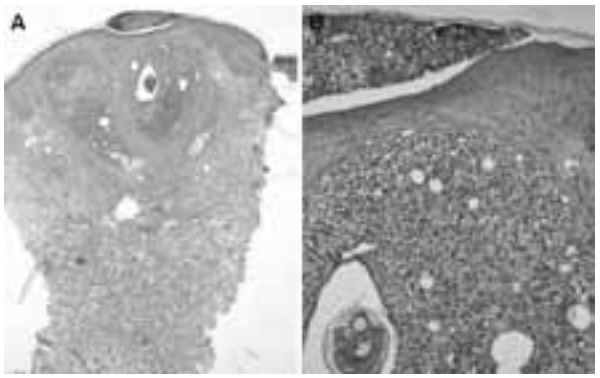
Dear Editor,

Drug rash with eosinophilia and systemic symptoms (DRESS) syndrome is a potentially life-threatening multisystem adverse drug reaction that must be recognised promptly to withdraw the causative drug. It is most commonly induced by aromatic anticonvulsants and antibiotics. In contrast, non-aromatic anticonvulsants are rare causes of DRESS. Therefore, we wish to add a case of valproic acid as the culprit drug inducing DRESS.

A 26-year-old man presented with acute liver failure with fever, a rash and lymphadenopathy. He had had an intracerebral bleed 5 months and an epileptic insult 1.5 months before presentation after which valproic acid 500 mg twice daily was prescribed. His remaining medication was: baclofen 500 mg three times daily, clemastine 2 mg twice daily and acetaminophen 1 g four times daily. He had not been abroad and had no history of animal/insect bites. As a child he had been vaccinated according to the national vaccination schedule for the Netherlands. He denied alcohol and intravenous drug use and unprotected sexual activities. His skin showed generalised papular-pustular exanthema, partly confluent to plaques on his chest, face and ears. Furthermore, he had facial oedema, yellow sclerae, erosions of his oral mucosa, petechial haemorrhages and lymphadenopathy at multiple sites. An enlarged liver without stigmata of chronic liver disease was observed. Laboratory results were compatible with severe liver dysfunction (prothrombin time 21.6 sec and albumin 27.6 mg/dl; aspartate aminotransferase 1219 IU/l, alanine aminotransferase 2800 IU/l, lactate dehydrogenase 763 IU/l, gamma glutamyl transaminase 216 IU/l, alkaline phosphatase 456 IU/l, and total and conjugated bilirubin 180 and 115 µmol/l). Acetaminophen and valproic acid levels were <2 mg/l (undetectable) and 47 mg/l (sub-therapeutic), respectively. Serological and microbiological assays did not yield an underlying cause for the liver failure. Ultrasonography revealed hepatosplenomegaly with hilar lymphadenopathy. A skin biopsy of a pustule demonstrated extensive superficial and deep infiltrates with eosinophils and lymphocytes and folliculitis (*figure 1A and B*). Valproic acid was replaced by levetiracetam and acetaminophen was stopped. Prednisone was started and within two days the cutaneous symptoms improved substantially and the PT shortened. After six weeks all symptoms had completely resolved, laboratory tests were normal and the prednisone was discontinued after 15 weeks.

This case report describes valproic acid-induced DRESS syndrome with fulminant liver failure. Valproic acid, a non-aromatic anticonvulsant, has not been described before as the culprit drug in DRESS. Furthermore, this report stresses the importance of a complete drug history and the need for clinicians to be aware of the delayed onset and the association between DRESS syndrome and liver failure.

Figure 1. *Histological images of a skin biopsy of a pustule*



Detail of epidermis showing extensive subcorneal en perifollicular infiltration of eosinophils and lymphocytes. Haematoxylin and eosin stain. Original magnification x 2 (A) and x 10 (B).

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2. Kaplan NM. Clinical Hypertension. 7th ed. Baltimore: Williams & Wilkins; 1998.
3. Powell LW, Isselbacher KJ. Hemochromatosis. In: Braunwald E, Fauci AS, Kasper DL, et al., editors. Harrison's Principles of Internal Medicine. 15th edition. New York: McGraw-Hill; 2001. p. 2257-61.

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