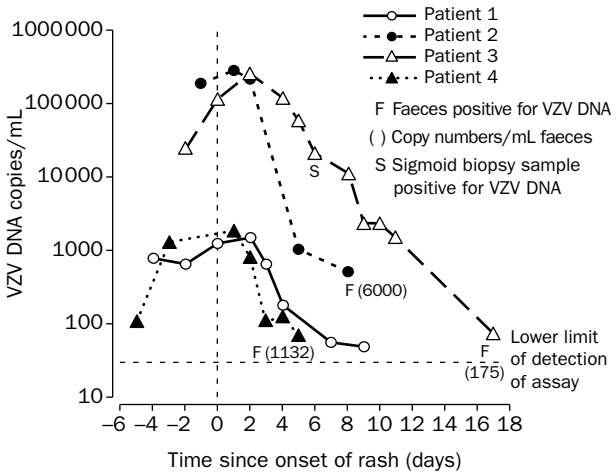

Varicella zoster virus (VZV) DNA load in blood by day since onset of cutaneous manifestations of herpes zoster

Patients 1–3 started acyclovir treatment at day 0, patient 4 at day –2. The lower limit of detection of the assay in serum was: 20 copies/mL; whole blood: 80 copies/mL.

3, 4) specimens, which had been obtained for other purposes before and after the onset of the skin eruption. VZV DNA could readily be detected in blood before the onset of cutaneous signs in all patients (figure). Maximum concentrations of viral DNA at this time ranged from 800 to over 180 000 copies/mL (median 10 500 copies/mL), increasing to 1270–270 000 copies/mL (median 59 500 copies/mL) upon cutaneous eruptions. These are quantities we had previously only observed in patients with chickenpox or disseminated cutaneous herpes zoster.[5] Considering the high concentrations, it is likely that VZV DNA would also have been detectable at earlier timepoints than tested. After 2–4 days of antiviral treatment VZV DNA concentrations declined rapidly in all patients (figure), which coincided with marked clinical improvement.

In three patients faecal specimens obtained during treatment were also analysed, and revealed quantities of VZV DNA in excess of the concentrations in blood at that time, which lends support to gastrointestinal involvement of VZV infection (figure). Moreoever, endoscopic examination was repeated after 4 days of antiviral treatment in patient 3, and showed multiple ulcerations in the oesophagus as well as widespread vesiculopapular lesions in the sigmoid colon. Although immunohistochemical staining of these lesions was negative for VZV, PCR analysis of biopsy samples clearly showed the presence of VZV DNA.

The clinical presentations in our patients are very similar to previous reports of visceral herpes zoster.[1–4] The high viral DNA loads in blood in the absence of skin eruptions and the detection of VZV DNA in faeces strongly suggest that herpes zoster indeed involved the viscera. Although strong evidence of visceral disease could only be provided in one patient, it is likely that the observed oesophagitis and raised liver enzymes in two other patients were also secondary to VZV infection.

We conclude that PCR detection of VZV DNA in blood, and perhaps faeces, can enable diagnosis of visceral herpes zoster in the absence of skin lesions, thereby preventing a potentially devastating delay in starting adequate antiviral treatment. In addition, quantitative VZV PCR may aid in monitoring effectiveness of treatment. Although prospective population-based studies are required to verify the sensitivity, specificity, and positive predictive value of such an approach, we urge clinicians to consider the possibility of early diagnosis of visceral herpes zoster by PCR when admitting BMT recipients, or other severely immunocompromised patients, who have unexplained abdominal pain.

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Departments of Medical Microbiology (M D de Jong MD, J F L Weel MD, R Boom PhD, P M E Wertheim-van Dillen MD)**, and Haematology** (M H J van Oers MD)**, Academic Medical Center, University of Amsterdam, Postbox 22660, 1100 DD Amsterdam, Netherlands**

Correspondence to: Dr Menno D de Jong (e-mail: m.d.dejong@amc.uva.nl)

Degradation of albumin in meningococcal sepsis

Philip C Holland, Stephen W Hancock, Donald Hodge, Douglas Thompson, Sue Shires, Stuart Evans

We postulate that the proteolytic degradation of albumin into fragments could link the rapidity of the shock, rash, and hypocalcaemia associated with meningococcal sepsis. We examined urine of children with meningococcal disease and urine from control children with no sepsis and found albumin fragments of about 45 kDa, 25 kDa, and less than 20 kDa only in the urine of children with meningococcal sepsis and associated purpura. Exogenous or endogenous proteases, or both, may be released in severe meningococcal sepsis and, in association with an inadequate antiprotease response, result in albumin degradation. This may be a contributory factor to the rapid shock, hypocalcaemia, and rash seen in meningococcal sepsis.

To the clinician managing meningococcal sepsis it is the rapidity of the shock, the development of the rash, and the metabolic derangement, particularly hypocalcaemia, which are the distinguishing hallmarks of this disease. The shock is attributed to the massive release of lipopolysaccaride (endotoxin) from *Neisseria meningitidis* and activation of inflammatory pathways resulting in loss of endothelial negative charge and leakage of whole albumin into the interstitium—the capillary-leak syndrome.[1] The development of the rash has been attributed to the increased procoagulant state associated with activation of inflammatory, thrombotic, and fibrinolytic pathways. Hypocalcaemia occurs in 70% of patients with severe meningococcal sepsis and the aetiology is open to debate

Patients	Bands degraded albumin	Calcium (mmol/L)	Rash	Micro-biology	Survival
Meningococcal septicaemia	29/30	1·3–2·4	30/30	17/30*	28/30
Meningococcal meningitis	0/2	2·3	0/2	2/2	2/2
Control	0/7	Not known	0/7	0/7	7/7

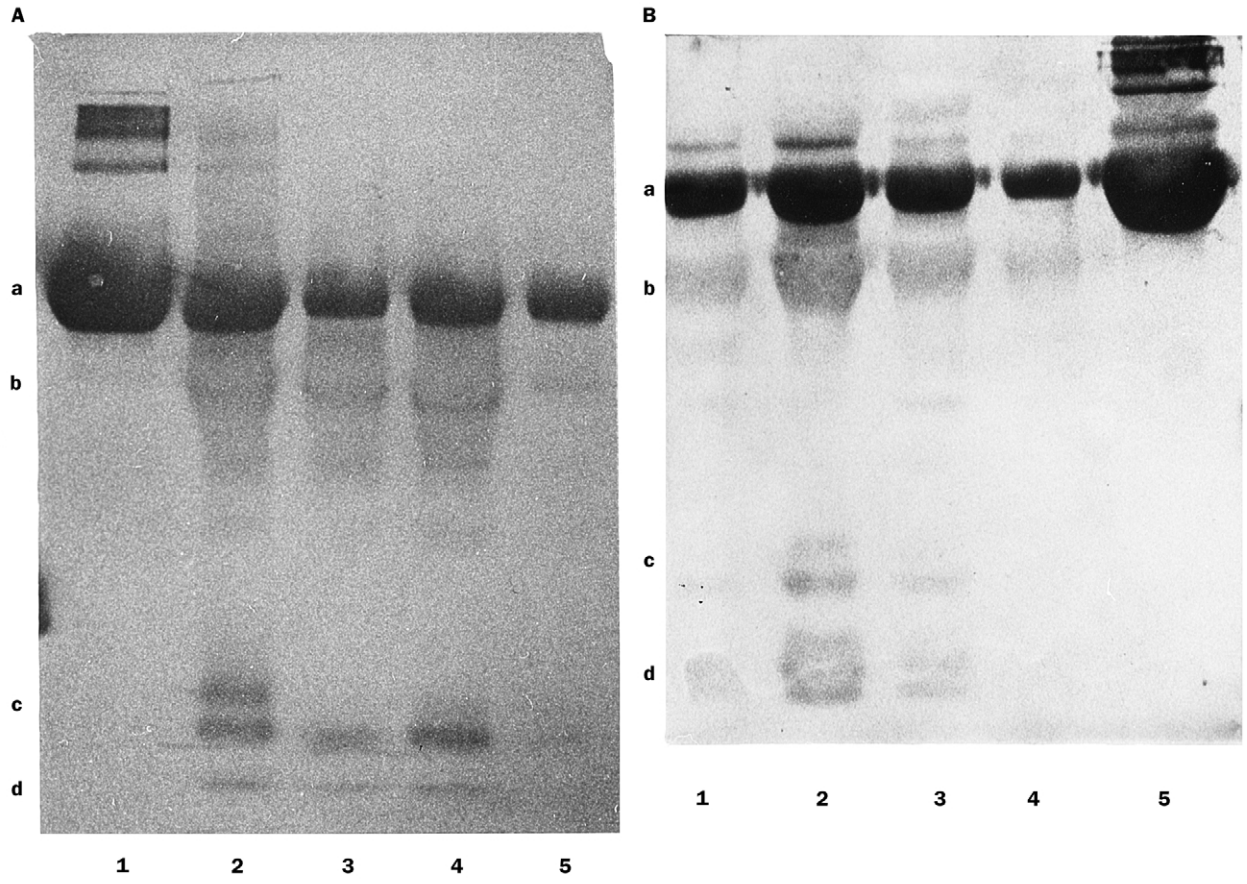
GMSPS=Glasgow Meningococcal Sepsis Prognostic Score. *Positive microbiology result either culture or polymerase chain reaction (PCR). PCR was not available in the first 10 patients.

Clinical characteristics

because there is no satisfactory explanation.[2] We question whether present explanations for the rapidity of the shock, rash, and hypocalcaemia are complete and suggest that a satisfactory hypothesis should link these three features. We postulate that the proteolytic degradation of albumin into fragments might provide such an explanation. The rapid loss of albumin fragments through the endothelium together with capillary leak would result in rapid shock. The loss of calcium together with the albumin fragments would contribute to the hypocalcaemia, and calcium leaking into the interstitium would result in acute calciphylaxis with purpura.[3]

We tested this hypothesis by examining urine of children with meningococcal disease and urine from control children with major injury, but no sepsis, admitted to the Yorkshire Paediatric Intensive Care Unit, Leeds, UK (table). Patients were categorised as having meningococcal sepsis with purpura and shock or meningococcal meningitis without purpura. Urine samples were collected within 24 h of admission and in five children serial samples were obtained. The urine samples were dialysed and put through sodium dodecyl sulphate polyacrylamide gel electrophoresis and the protein was electrophoretically transferred from the gel on to a nitro-cellulose membrane. Albumin transferring to the membrane was located using a sheep polyclonal antibody to human albumin conjugated with horseradish peroxidase (Dako Ltd, High Wycombe, UK). A random sample was checked with a second antibody to human albumin (Serotec, Oxford, UK) although no difference was found. These Western blots were scanned using a charged-coupled device camera to semiquantify any whole albumin and albumin fragments present.

As well as whole albumin (66 kDa), bands of degraded albumin were found, at about 45 kDa, 25 kDa, and less than 20 kDa in 29 of 30 children with meningococcal sepsis and rash. Although whole albumin was found, no albumin fragmentation was seen in the control patients, the two children who had meningococcal meningitis without a rash, and in one patient with meningococcal sepsis (table). In one patient with albumin fragments, ascitic fluid taken at the time of insertion of a dialysis catheter was available and this showed similar albumin degradation. The albumin fragments accounted for 23–46% of the total albumin lost in the urine from the



Western blot, and staining with antibody to human albumin, of urine from two patients with meningococcal sepsis (A); Western blot of urine taken serially from one patient with severe meningococcal sepsis (B)

HAS=human albumin solution, GMSPS=Glasgow Meningococcal Sepsis Prognostic Score. A. Lane 1: HAS alone. Lanes 2–4: patient with severe meningococcal sepsis (GMSPS=13) on admission (lane 2); after 24 h (lane 3); and after 36 h (lane 4) Lane 5: patient with milder meningococcal sepsis (GMSPS 8) on admission. a=at 66 kDa (whole albumin); b=fragments at 45 kDa; c=25 kDa; d=≤20 kDa. B. Lane 1: 14 h. Lane 2: 24 h. Lane 3: 33 h. Lane 4: 57 h. Lane 5: HAS alone. a=66 kDa (whole albumin); b=fragments at 45 kDa; c=25 kDa; d=≤ 20 kDa.

mild to the severe cases, respectively (figure A), with fragments of albumin disappearing by 3 to 4 days after initial presentation when the patient was improving (figure B). 4% human albumin solution (HAS) and fresh frozen plasma (FFP) tested separately did not contain fragmented albumin. There was a significant inverse relationship between the Glasgow Meningococcal Sepsis Prognostic Score (GMSPS) and lowest total plasma calcium concentration ($p<0{\cdot}01$, $r=-0{\cdot}57$).

To test the hypothesis outlined above, this preliminary study was designed to examine whether albumin is degraded into multiple fragments in meningococcal sepsis. The concentration of albumin in the urine has been used in the past to give some estimate of severity of the capillary leak; we therefore associated the amount of albumin found within the fragments to that of whole albumin. Although whole albumin was found in all patients with sepsis, bands of degraded albumin were only detected in the patients with sepsis and a rash. The quantity of degraded albumin in the bands was associated with severity. The presence of albumin fragments in the one sample of peritoneal fluid available during the study suggests that albumin fragmentation is not associated with leakage of albumin through the kidney itself. Because all patients with sepsis required either HAS or FFP for resuscitation we tested these fluids for presence of albumin fragments in case albumin fragments were introduced during the manufacturing process. Such contamination was not found. Although we were only able to measure total plasma calcium in this study, another study has shown a close correlation between total and ionised calcium.[2] Bound calcium (total minus ionised calcium) was also found to be low, particularly in those children with the lowest calcium, a reflection of albumin loss either as whole albumin or as fragments.

The complete genomes for *N meningitidis* serogroup A and B have been described and are known to contain genetic sequences compatible with serine proteases. Proteolytic cleavage of albumin is described with the albumin molecule unfolding at low pH, compatible with the environment of the peripheral microcirculation during severe shock. This may explain why the rash primarily involves the capillaries where there is both a low pH and meningococci trapped within the circulation.[4,5] Our study confirms the presence of albumin fragmentation and therefore suggests that our hypothesis should be examined in more detail because it may provide an explanation for the severe sequellae associated with meningococcal sepsis.

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Departments of Paediatrics, Clarendon Wing (P C Holland MBBS, S W Hancock MBChB, D Hodge MBChB)**, and Clinical Biochemistry and Immunology** (D Thompson PhD, S Shires BSc)**, General Infirmary at Leeds, Leeds LS2 9NS, UK; Molecular Epidemiology Unit, University of Leeds, Leeds** (Stuart Evans PhD)

Correspondence to: Dr Philip Holland
(e-mail: hollandp@ulth.northy.nhs.uk)

Risk of trisomy 21 in offspring of patients with Klinefelter's syndrome

S Hennebicq, R Pelletier, U Bergues, S Rousseaux

Intracytoplasmic sperm injection (ICSI) has given some patients with Klinefelter's syndrome (ie, men with an XXY sex-chromosome profile) the chance to become fathers, but the genetic makeup of the spermatozoa used for the injection is a concern. We studied the segregation of the sex chromosomes and chromosomes 1 and 21 by multicolour fluorescence in-situ hybridisation in a patient with non-mosaic Klinefelter's syndrome who was a candidate for ICSI. As other workers have found, we saw a higher rate of 24,XX and 24,XY spermatozoa in the patient than in controls. However, we also found a much higher frequency of disomy 21 in the spermatozoa of this patient than in controls (6·2 *vs* 0·4%). Any child conceived by ICSI using this man's sperm will thus have a proportionally higher risk of trisomy 21.

Klinefelter's syndrome, which is characterised by the genotype 47,XXY, and which affects about one in 1000 men, is the most frequent genetic cause of human male infertility (4·6%) and occurs in 12% of azoospermic men. Since some patients with Klinefelter's syndrome can become fathers by means of in-vitro fertilisation (IVF) combined with intracytoplasmic sperm injection (ICSI), the safety of the technique, including the genetic makeup of the injected spermatozoa, has become a major concern. Cytogenetic studies on sperm suggest an increased frequency of 24,XY spermatozoa in patients with Klinefelter's syndrome,[1] meaning that male offspring are at increased risk of Klinefelter's syndrome themselves. The risk of autosomal abnormalities in such offspring has not yet been fully assessed.

We studied the segregations of chromosomes X, Y, 1, and 21 by multicolour fluorescent in-situ hybridisation (FISH) in a 34-year-old patient with non-mosaic Klinefelter's syndrome who was a candidate for an ICSI procedure, and in three healthy controls. The DNA probes specific for chromosomes X, Y, and 1 (plasmid probes pXBR2, pHY2.1, and pUCI.77, respectively) were cohybridised in three-colour FISH experiments as described elsewhere (details available from the authors). Chromosomes 1 and 21 (YAC probe 745HII specific for 2lq22.2) were codetected in dual-colour FISH experiments.

Our findings were similar to those of a previous study:[1] the frequencies of 24,XY and 24,XX hyperhaploid spermatozoa in the patient with Klinefelter's syndrome were substantially higher than in the controls (table). Thus the theoretical risk of fathering a 47,XXY or 47,XXX child after successful IVF ICSI was higher too. However, we also found a much higher frequency of disomy 21 in the spermatozoa of our patient than in our controls (table). This finding implies a proportionally higher risk of trisomy 21 in a child conceived by ICS1.

The effect of a structural or numerical chromosomal abnormality on the segregation of the other chromosomal pairs during meiosis (the interchromosomal effect) has been investigated previously in individuals with balanced translocations, and in men with sex-chromosome abnormalities. No evidence was found of a clear interchromosomal effect, except in the study by Blanco and colleagues, who found a substantially higher rate of disomy 21 in the spermatozoa of a t(3;15) carrier than in controls (1·90 *vs* 0·37 %).[2] The most likely reason for the high rate of disomy 21 in our XXY patient is that the abnormal sex chromosome content disturbs the meiotic segregation of some chromosomal pairs, possibly via the presence of an abnormal