Meningococcal bacterial DNA load at presentation correlates with disease severity

S J Hackett, M Guiver, J Marsh, J A Sills, A P J Thomson, E B Kaczmarski, C A Hart

Methods: Meningococcal DNA quantification was performed by the Taqman PCR method on admission and sequential blood samples from patients with MCD. Disease severity was assessed using the Glasgow Septicaemia Prognostic Score (GMSPS, range 0–15, severe disease ≥8).

Results: Median admission bacterial load was $1.6 \times 10^4$ DNA copies/ml of blood (range $2.2 \times 10^4$ to $1.6 \times 10^5$). Bacterial load was significantly higher in patients with severe ($8.4 \times 10^4$) compared to mild disease ($1.1 \times 10^4$, p = 0.018). This difference was greater in septicemic patients (median $1.6 \times 10^4$ versus $9.2 \times 10^3$, p = 0.001). Bacterial loads were significantly higher in patients that died ($p = 0.017$). Admission bacterial load was independent of the duration of clinical symptoms prior to admission, with no difference between the duration of symptoms in mild or severe cases (median, 10.5 and 11 hours respectively). Bacterial loads were independent of DNA elimination rates following treatment.

Conclusion: Patients with MCD have higher bacterial loads than previously determined with quantitative culture methods. Admission bacterial load is significantly higher in patients with severe disease (GMSPS ≥8) and maximum load is highest in those who die. Bacterial load is independent of the duration of clinical symptoms or the decline in DNA load.

METHODS

All children admitted to the Royal Liverpool Children’s Hospital with a diagnosis of probable and possible MCD between January 2000 and January 2001, were seen at admission. Clinical history and examination as well as the results of laboratory investigations were recorded on a previously validated laboratory information system. The meningococcal DNA detected will obviously represent killed as well as viable meningococci, but will be directly related to bacterial antigen load.
Meningococcal DNA load and severity

Figure 1  Correlation between admission bacterial loads and maximum GMSPS.

single copy capsular gene (ctrA) common to all N meningitidis serogroups. Real time PCR detection by Taqman (4–5 hours) provides accurate quantification of bacterial load over a range of 10^3 to 10^8 copies per ml. The quantification of DNA for all samples from individual patients was performed on a single plate using the same standards. The intra-assay variability was minimal as shown by CMV quantification which uses the sample DNA extraction and Taqman method, with significant variation (maximum 0.5 log-fold difference) only seen at low DNA concentrations (10^3 to 10^4). The bacterial load is expressed as genome copies per millilitre of blood. The lower detection limit is 10^4 copies per ml because only microlitre amounts of clinical material are assayed in each test. As each bacterium contains only a single capsular gene, DNA load equates to bacterial load. For this study, we use the terms interchangeably.

This study was part of a larger study of the pathogenesis of MCD and was approved by the Local Research and Development and Ethics Committees.

RESULTS

The median bacterial load in the 51 patients at admission was 1.6 x 10^7 DNA copies/ml of blood (range 2.2 x 10^6 to 1.6 x 10^8). Patients with severe disease had significantly higher meningococcal DNA loads (n = 27, median 8.4 x 10^7, range 2.2 x 10^6 to 1.6 x 10^8) than patients with mild disease (n = 24, median 1.1 x 10^7, range 2.5 x 10^6 to 1.3 x 10^7) (Mann–Whitney, p = 0.018). The disparity between patients with mild and severe disease increased when patients with primarily septicaemia (without major symptoms and signs of meningitis) were looked at separately (median 9.2 x 10^6 versus 1.6 x 10^7, p < 0.001). This association of bacterial load and disease severity was also shown, as a continuous positive correlation, against all GMSPS values (Spearman’s rank correlation 0.5, p < 0.001; fig 1).

In seven patients, DNA load increased marginally within 16 hours of admission, to a maximum of 1.8 x 10^8 and then decreased. These patients had severe disease (GMSPS >8) and their clinical conditions deteriorated notably after admission. The maximum bacterial load was significantly higher in the two patients that died (3.1 x 10^8 and 1.8 x 10^8) compared to survivors (Mann–Whitney, p = 0.017). When the maximum bacterial load for survivors and non-survivors in the severe group were compared, there was a trend towards difference that did not quite reach significance (p = 0.055).

The median duration of clinical symptoms (usually fever) prior to attendance at hospital or treatment: temperature, vomiting, drowsiness/lethargy, macular or petechial rash, or time since onset of prodromal respiratory type illness (Spearman’s rank correlation, p > 0.43).

Bacterial DNA was detectable up to 4.5 days following admission in sequential EDTA samples (range 22–109 hours, median 40). The decrease of DNA load in these sequential samples was highly variable. Patients who had very similar clinical pictures, who received comparable treatments, showed a notable difference in DNA clearance rates. The rate of decline in meningococcal DNA load over time was assessed using linear regression analysis (fig 2). There was no association between disease severity (Spearman’s correlation, p = 0.66) or admission bacterial load (Spearman’s correlation, p = 0.8) and the rate of DNA decline. One patient who did not have an immune deficiency during this study, had two infective episodes both caused by group B N meningitidis. The DNA elimination rates from both episodes, the first principally septicaemia and the second meningitis, were identical.

DISCUSSION

We have shown that bacterial load at admission is far higher than had previously been appreciated in MCD when assessed by quantitative culture methods. Loads were also higher than has been shown in infections with other bacteria such as H influenzae or S pneumoniae. This is consistent with the results that bacteria DNA concentrations measured by Taqman PCR were up to 2-log fold higher than predicted when samples were inoculated with known quantities of colony forming meningococci from culture (M Guiver, personal communication).

The range of load measurements accords with recent findings that cytokine release from monocytes in vitro increases on stimulation by whole meningococci at concentrations from 10^6 to 10^7 bacteria/ml. At bacterial concentrations of 10^7, cytokine release from monocytes stimulated by both LOS expressing and LOS deficient bacteria were identical, indicating an LOS independent stimulation pathway. It was not explored whether bacterial concentrations greater than 10^8 would induce a greater LOS independent cytokine response, which could ultimately influence outcome.

The association of bacterial load with disease severity was predictable but had not been shown in MCD before. This association is strongest if patients with predominantly meningitis are excluded from the analysis (illustrated by a patient with meningitis where the load in the blood was 3 x 10^8 compared to 5 x 10^6 in CSF and in the patients that died).

Several patients showed a transient increase in meningococcal DNA concentrations after admission and treatment. This presumably indicates that in some patients meningococci are sequestered in either peripheral white cells or on endothelial surfaces and then released into the circulation following antibiotic therapy.
We showed that disease severity and initial bacterial load at admission are independent of the length of clinical symptoms. Children with MCD are described as being “completely well” until they develop their first clinical symptoms, usually a raised temperature. The lack of a prolonged prodromal illness and the often abrupt onset of clinical symptoms possibly means that symptoms occur promptly following the initial bacteraemia. The doubling time for meningococci in culture medium is approximately 20–30 minutes. Under “ideal” conditions, therefore, the bacterial loads measured in our patients with severe disease (10⁸ to 10¹⁰) could be achieved in 10–15 hours, corresponding closely to the duration of symptoms prior to admission. It is probable that the hosts’ response to infection is the major component determining multiplication rates for meningococci, as the same strains of meningococci that circulate in any population cause severe disease in only the minority of patients who encounter them. Recent work has shown that meningococci liberate increased concentrations of lipopolysaccharide (LOS). As endotoxin assays are labourious, the availability of a method that can rapidly measure bacterial load (presently 4–5 hours but new LightCycler PCR technology can yield results in 30–60 minutes) may in future be used as a prognostic marker to aid selection of appropriate specific therapies for treating MCD. What is unclear is whether more “virulent” meningococci liberate increased concentrations of LOS. Future work is needed to compare endotoxin concentrations directly with bacterial loads.

ACKNOWLEDGEMENTS

This study was supported by funding from the Meningitis Research Foundation, Meningitis Mersyside, and the Johanne Holly Research Fund. I would like to thank Dr Ian Campbell for his statistical advice throughout this project.

Authors’ affiliations

S J Hackett, J A Sills, A P J Thomson, Institute of Child Health, Alder Hey Children’s Hospital, Eaton Road, Liverpool L12 2AP, UK
M Guiver, J Marsh, E B Kaczmarski, PHLS Meningococcal Reference Unit (MRU), Withington Hospital, Manchester M20 2LR, UK
C A Hart, Department of Medical Microbiology, Duncan Building, Liverpool University, Daulby Street, Liverpool L69 3GA, UK

REFERENCES

Meningococcal bacterial DNA load at presentation correlates with disease severity


Arch Dis Child 2002 86: 44-46
doi: 10.1136/adc.86.1.44

Updated information and services can be found at:
http://adc.bmj.com/content/86/1/44

These include:

References
This article cites 14 articles, 1 of which you can access for free at:
http://adc.bmj.com/content/86/1/44#ref-list-1

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/