

Invited paper

A clinical laboratory approach to severe sepsis

The changing role of laboratory medicine in clinical decision support during management of septicaemia.

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Abstract

Sepsis has a different meaning to the surgeon, physician and intensivist than the clinical microbiologist. Understanding the difference between a front line clinician's diagnosis and determination of the microbial cause of severe sepsis is the key to unlocking the contribution of the clinical laboratory to critical decision support for septicaemic patients. Correct and effective use of blood culture, other culture and non-culture based methods of determining aetiology, followed by monitoring progress in severe sepsis are time-critical support measures for treatment decisions. Nucleic acid amplification techniques and MALDI-TOF mass spectrometer use have sped up the interval between blood culture inoculation and determination of a definitive aetiology. However, the aetiology can only rarely be determined soon enough to direct presumptive antibiotic choice in severe sepsis. More often, presumptive antibiotic and supportive care decisions have to be made with guidance from guidelines, clinical trial results and local laboratory-derived epidemiology. The contribution of the clinical microbiology laboratory is therefore more often in refinement of antibiotic treatment and the monitoring of progress. Until emerging laboratory technology has more to offer in the immediate assessment of severe sepsis, the clinical microbiologist will continue to play a mainly supportive role as a member of a multidisciplinary team. This is likely to change as a range of systems biology tools start to make an impact on the clinical laboratory.

The meaning of sepsis.

Septicaemia is a slippery word. It has a range of meanings depending on who, where and when it is used. It is therefore useful to distinguish between the clinical sepsis familiar to surgeons and general physicians, the sepsis syndrome seen in the critically ill and the clinical manifestations of laboratory confirmed bacteraemia, viraemia, fungaemia or parasitaemia familiar to clinical microbiologists. Of course, the patient with an established febrile illness

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may have all of these at once. Consequently, their condition will be described by different medical specialists according to their respective specialist skills – from the salient clinical features and derangements in physiological functions to the demonstrable features of infective agents. Familiarity with the methods surgeons, general physicians, anaesthetists and medical microbiologists use to reach their distinctive but complementary conclusions will help to reduce misunderstanding and foster the interdisciplinary management so important for the patient with severe sepsis.

The difference between diagnosis and aetiology.

Clinical microbiology has a tendency to overstep its boundaries when contributing to the care of the septic patient. The working diagnosis is made by the front line clinician who may be a physician, surgeon or intensivist. They will often arrive at a differential diagnosis, which may be very broad in the case of a febrile patient, and initiate a series of laboratory investigations. It is only rarely that a clinical microbiologist makes the initial clinical diagnosis *de novo* and has an opportunity to confirm their initial suspicion by following through with a series of imaging and laboratory investigations.

The principal role of the clinical microbiology laboratory is to assist in a clinical decision-making process. Clearly, the laboratory's strength is in determining the identity of the causal agent of infection (the aetiology, sometimes referred to as the aetiological diagnosis) and determines critical features that guide choices of antimicrobial therapy and infection control procedures. A simple analogy is that as the clinical microbiology laboratory adds critical detail to the front line doctor's initial assessment and plan, adjectives and adverbs are added by the laboratory to the bare bones of nouns and verbs used by the attending physician. Thus septicaemia becomes staphylococcal septicaemia, meningitis becomes meningococcal or pneumococcal meningitis and so on. In summary, the –itis of meningitis is transformed to an –osis of meningococcosis.

An interesting paradox is that when the clinical microbiology laboratory describes its role and mission more accurately, it is better able to perform its core set of tasks. Instead of trying to construct a diagnosis, the clinical laboratory contributes a small but critical detail to the overall clinical decision-making process: a “micro-gnosis”. The micro-gnosis is the identified necessary and sufficient microbial cause of septicaemia; its aetiology. The contribution of other pathology disciplines to this process in septicaemic patients will be covered briefly later in this short review.

Timelines

Time is the enemy of the patient with severe sepsis. Early, decisive intervention is critical. The problem is that it is least possible to be certain about a definitive course of antimicrobial and supportive therapy in the earlier stages of sepsis. We know from studies on bacteraemic patients that active investigation on the day of admission is associated with a marked reduction in the crude mortality rate compared to delayed investigation on day two or later.¹ This is not to suggest that laboratory investigations have a direct therapeutic effect. It is more likely that early investigation is a surrogate marker for prompt and presumptive antimicrobial therapy. In the same series we noted that patients with community-acquired septicaemia who were transferred directly to intensive care or the high dependency unit did much better than those transferred to general wards. The introduction of a four-hour rule² to encourage prompt clinical assessment of emergency department patients has yet to be shown to have a

significant effect on preventing progression from uncomplicated to severe sepsis, but needs to be balanced with the risk of losing critical early results during the transfer to a general ward.

Clinical decision support

The question of how best to provide support to the front line team caring for the patient with severe sepsis is a complex one.³ This will differ according to the specialist skills, services and supplies available on site. At the very start of our long-term development process, we run a critical decision path analysis of septicaemia in our hospital. The specific details are relevant only to our own hospital service, but the senior clinical microbiologist can perform this analysis in collaboration with general physicians and surgeons in any hospital. Its outcome is a specific appreciation of key steps in the clinical decision-making process surrounding septicaemic patients. This is a valuable tool for directing the support efforts of the clinical laboratory, and improving the flow of time-critical information.

Blood culture methods

Not all patients with severe sepsis have an infection and bacteria are not the only cause of those who do have an infection. However, all patients with severe sepsis should have a peripheral blood culture. The minimum recommended is two sets of bottles i.e. 2x aerobic and 2x anaerobic bottles. Subsequent sets of cultures yield little additional clinically useful results.⁴ The maximum necessary for a single septic episode is four sets (i.e. 8 bottles) after which little additional information is obtained by repeated sets. The exception is the investigation of subacute infective endocarditis. If severe sepsis is bacterial in aetiology and the patient has yet to receive antibiotic treatment, the concentration of bacteria in the peripheral blood is often so high that cultures will be recognisably positive after only a few hours in the laboratory incubator. However there is an important caveat. We have only rarely encountered a positive adult blood culture inoculated with less than 3.0mL blood. Most commercial adult blood culture bottles are designed to cope with between 8-10mL inoculated blood. Smaller volumes will risk missing a low-concentration bacteraemia. Higher volumes may slow down bacterial growth because there will not be enough anticoagulant to prevent microclots forming. Our practice is to encourage junior doctors to avoid filling the blood cultures last in a series of routine investigations with a tiny volume of residual blood. Volumes as small as 1.0mL shared between two bottles reduce the chances of successful culture, and maximise the risk of contamination by skin bacteria (Table 1). We strongly recommend matching regional cultures that target potential localised sources of systemic infection (e.g. urinary tract, lungs, skin and soft tissues) collected at the same time. Conversely, blood cultures should be collected from any patient with severe sepsis who is being considered for urine, sputum or wound swab collection.

Speeding up clinical microbiology

The battle against the clock for patients with severe sepsis is not helped by reliance on culture-based methods. In our centre, the initial Gram stain results take a median of around 12 hours after culture collection. Definitive identification usually takes 24-48 hours and antibiotic susceptibility determination longer still. That is, if we rely on culture-based methods. Improved blood culture bottle inoculation technique will make small improvements and a higher proportion of blood cultures generate positive results in centres that train staff in best blood culture practice. Conventional culture methods alone cannot shorten the overall time to definitive identification of the causal infective agent to less than 12-24 hours without

Table 1 : Optimal blood culture procedure for adult patients with suspected septicaemia.

Procedure stage	Comment
1. Patient selection	Hospital patients with fever, hypothermia, raised pulse, respiratory rate or other features of sepsis <u>Or</u> focal infection plus fever.
2. Body site	Peripheral vein phlebotomy by preference over existing intravenous cannula; forearm preferred to other sites
3. Sample collection kit assembly	Prepare with bottles, syringes, needles, gloves, skin disinfectant, drapes away from patient bedside
4. Skin preparation	Wash and glove hands THEN apply iodophor or chlorhexidine at phlebotomy site <u>after</u> vein palpation. Allow to dry, maximising disinfectant effect
5. Bottle preparation	Ensure bottles labelled with patient identifier, date and time. Pair of aerobic and anaerobic high fill bottles. Remove lid cover. Disinfect rubber cap. Allow disinfectant to dry.
6. Venesection	20mL blood per pair of bottles. If with other samples, blood culture should come FIRST to avoid contamination.
7. Bottle Inoculation	Active dispensing of 8-10mL per high fill bottle. Avoid volumes < 3mL and > 12mL. Do not leave to fill passively under vacuum.
- order	Aerobic before anaerobic
- volume	8-10mL per blood culture set
- action	Active inoculation
- repeats	Second and further sets give diminishing yield except in infective endocarditis when 3 sets at intervals is standard
8. Laboratory request	Make sure patient identification matches bottles, indication for investigation stated, prior antibiotics noted
9. Transport	Dispatch bottles and request form together to start incubation as soon as possible. Delayed entry can result in false negatives
10. Other microbiology samples	Consider sampling specific sites e.g. BC from i.v. lines, MSU, sputum, wound swab
11. Antibiotic therapy	Start presumptive antibiotic therapy after cultures collected. Make sure first dose given
12. Result reconciliation	Initial results (Gram stain) can take 12-24hr from inoculation. Actively seek these results and subsequent identification, antibiotic susceptibilities

assistance from new laboratory methods. A variety of molecular methods have been used to speed up identification of the commoner causes of bacteraemic septicaemia including 16s and species-specific PCR assays, but these are still very much a work in progress.⁵ These will generate an identification in a few hours, shortening the time taken to achieve a micrognosis. In most centres the commonest cause of bacteraemia are the staphylococci. Early confirmation of *Staphylococcus aureus* bacteraemia and detection of methicillin resistance has been a high priority for clinical microbiology services in large teaching hospitals, making this one of the first molecular methods to gain widespread acceptance in hospital medicine, though its impact on clinical outcomes has been disappointing.⁶ Nucleic acid amplification tests such as PCR assays can be designed with a high degree of specificity, and have a sensitivity well above what is required for confirmation of blood culture contents. Multiple PCR assays are however an inefficient method for identifying a potentially vast range of causes of septicaemia. The most important advance in blood culture process of the last decade has been the introduction of mass spectrophotometers into clinical microbiology. Matrix-assisted laser-desorption time of flight (MALDI-TOF) mass spectrometry now provides a method for rapid identification of bacteria in up to 90% blood cultures.⁷ There are libraries containing thousands of different bacterial species' mass spectrophotometer patterns from which to make a rapid identification. The overall result of these nucleic acid and proteome enhancements to the clinical laboratory have been to shave around 24 hours from the time to

definitive identification. MALDI-TOF methods for rapid detection of antibiotic resistance are now coming into wider use. But despite these improvements, the clinical laboratory is still incapable of contributing much to initial decisions about antibiotic and supportive treatment of severe sepsis.

Presumptive therapy

The initial choice of antibiotic therapy relies largely on clinical guidelines, which in turn are based on a combination of clinical trial data and local epidemiology. Implementing agreed practice guidelines for the immediate management of sepsis have recently been shown to improve clinical outcomes.⁸ The microbiology laboratory's contribution is therefore the long term surveillance of antibiotic resistance patterns and contribution to local clinical trials. The patient with severe sepsis of unknown cause is liable to treatment with multiple antibiotics, many of which will become unnecessary when a micrognosis has been established. In the setting of severe sepsis, it is often necessary to consider the potential adverse effect of preferred intravenous antibiotics on other therapeutics agents, renal, hepatic, lung and haematopoietic function. Antibiotic choice may be restricted by more than acquired antibiotic resistance.

Supportive care

Clinical microbiology takes an aetiological focus on the clinical management of the septic patient. In the setting of severe sepsis, the support given to the patients organ systems is just as important a determinant of clinical outcome. A recent review of the relationship between the biochemistry underling the patient's redox potential and clinical outcomes in sepsis emphasises how difficult therapeutic manipulation of acute biochemistry can be.⁹ Putting this knowledge into action is a work in progress. The febrile patient usually has an increased fluid and oxygen requirement, but these must be managed carefully in patients with congestive heart failure, chronic renal failure and chronic obstructive lung disease. As the patient's condition deteriorates into severe sepsis, acute organic failure may alter the distribution of antibiotics, increase the risk of toxicity and reduce elimination of microorganisms and their toxins. As a rule of thumb, the more organ systems fail due to severe sepsis, the poorer is the prognosis.¹⁰ This explains the importance of organ systems support in severe sepsis, best achieved in an intensive care unit. At a simpler level, successful treatment of patients with sepsis in the setting of metabolic disease such as diabetes or chronic renal failure often depends on active treatment of the underlying metabolic anomaly which sepsis precipitates or exacerbates.¹¹ Glycaemic control is evidently an important adjunct to management of severe sepsis, and may be more important a determinant of acute outcome in non-diabetics.¹²

Clinical progress and outcomes

Clinical microbiology is concerned with more than the aetiology of severe sepsis. The progress of this condition either into multiple organ systems failure and death, or towards recovery, depends on the cooperation of a multidisciplinary group of specialists including clinical microbiologists and other pathologists. The clinical laboratory can assist with monitoring progress through haematological indices, acute phase reactants such as C-reactive protein, pro-calcitonin and other acute phase reactants,¹³ and the persistence of the causal agent of infection. The antimicrobial susceptibility pattern is a critical contributor to decisions on definitive antibiotic treatment, and possibly the need for enhanced infection control

measures. This information will also determine the choice of step-down therapy during the patient's defervescence.

New arrivals in the laboratory contribution to subsequent patient management are measures of bioburden or microbial load. The use of bacterial load as an indicator of response to therapy is still in its infancy.¹⁴ At present, truly quantitative measures are only widely used for viruses such as HIV,¹⁵ and to a lesser extent for severe *Plasmodium falciparum* malaria.¹⁶ The development of accurate quantitative standards for PCR assays will allow the clinical microbiologist to follow trends during the acute stages of severe sepsis; trends that may give early warning of escalation, or indication of response to treatment. Another laboratory innovation that could assist in critical decision making concerning the patient with severe sepsis is the measurement of circulating cell free DNA, which has been observed to correlate with the severity of sepsis.¹⁷ A recent Canadian study found measurement of circulating cell free DNA and other indicators of sepsis to assist in early prediction of disease severity and eventual outcome.¹⁸ This approach is in its early stages and has yet to develop into a reproducible clinical pathology assay.

Conclusion

Sepsis is one of the world's most common fatal infectious diseases. Yet it lacks the profile of mono-aetiology infections like malaria, tuberculosis and HIV-AIDS. The complex nature of the clinical challenge and its bewildering range of aetiological agents make it difficult to concentrate the effort of clinical support services. The clinical microbiologist has a coordinating role in the multidisciplinary management of patients with severe sepsis, and as a critical knowledge resource for the front line clinician.

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