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Review

Diagnosis and treatment of Panton–Valentine leukocidin (PVL)-associated staphylococcal pneumonia

M.S. Morgan *

Royal Devon & Exeter Foundation Trust, Barrack Road, Exeter EX2 5DW, UK

Abstract

Panton–Valentine leukocidin (PVL)-producing *Staphylococcus aureus* is emerging as a serious problem worldwide. Whilst usually causing skin and soft-tissue infections, particularly recurrent abscesses, there has in the last 10 years been an increase in the incidence of an associated devastating pneumonia affecting previously healthy young people and associated with a very high mortality. There are no evidence-based guidelines to consult for the management of PVL-associated staphylococcal pneumonia. The literature contains less than 100 cases, with widely differing antimicrobial therapies and the occasional use of other adjunctive therapies such as intravenous immunoglobulin, activated protein C and extracorporeal membrane oxygenation. This literature review focuses on the salient features of diagnosis and management, with particular attention to the choice of antimicrobials.

Keywords: Panton–Valentine leukocidin; Necrotising pneumonia; Community-associated methicillin-resistant *Staphylococcus aureus*; CA-MRSA

1. Introduction

Before 1950, staphylococcal pneumonia was uncommon, rarely seen except during influenza epidemics [1]. With appropriate antibiotic therapy, mortality was low, usually <3% [2]. However, since 1999 *Staphylococcus aureus* strains producing Panton–Valentine leukocidin (PVL) have been associated with a particularly vicious form of necrotising pneumonia [3] characterised by abscess formation, cavitation, haemorrhage, necrosis and a mortality approaching 75% [4].

2. Panton–Valentine leukocidin (PVL)

PVL, named after the authors of the paper published in 1932 [5], is encoded by two genes, *luk-S-PV* and *luk-F-PV*, and is transferred between heterogeneous strains of *S. aureus* by bacteriophages [6]. Five years ago, <2% of invasive clinical isolates of *S. aureus* submitted to a reference laboratory in the UK produced PVL [7].

Whilst the main diseases caused by PVL-positive strains are sporadic cases and small outbreaks of necrotising skin and soft-tissue staphylococcal infection, increasingly severe pneumonias with a high morbidity and mortality are being recognised.

One of many toxins produced by *S. aureus*, PVL is structurally similar to gamma-haemolysin, comprising two subunits (F and S). These dimeric toxin molecules are termed 'synergohymenotropic', binding to and assembling on the neutrophil membrane into an octomeric structure, opening calcium channels by causing pore formation. Exocytosis of granules and production of interleukins and other inflammatory mediators from neutrophils causes local vasodilatation, chemotaxis and additional neutrophil recruitment. Secretion of degradative enzymes and generation of superoxide ions promote tissue necrosis [8].

Clones of PVL-producing methicillin-resistant *S. aureus* (MRSA) are now spreading rapidly throughout the world. Although France, America and Australia have reported cases since 1999, the first recognised British case of PVL-associated pneumonia was in London in 2003 [9].

* Present address: Medical Microbiology, Old Pathology Laboratory, Church Lane, Exeter EX2 5AD, UK. Tel.: +44 1392 402 970.
E-mail address: marina.morgan@rdeft.nhs.uk.

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3. Incidence of PVL-associated pneumonia

The true incidence of PVL-associated pneumonia is unknown, since the number of cases published is likely to be an underestimate and cases may go unrecognised. Molecular testing for the presence of PVL genes is not routinely performed in hospital laboratories, and clinical suspicion has to be aroused to trigger sending the isolate to a reference laboratory for toxin testing.

On reviewing the literature, 71 cases of fatal pneumonia due to PVL-associated staphylococcal infection have been reported, mostly as case reports or small series [10–40]. The largest series was in the seminal paper by Gillet et al. [4] who reported 12 deaths. Forty-three survivors have been reported in total [4,17,29,35–37,41–58], resulting in an overall fatality rate of 62% in these reported cases.

Since Gillett et al.'s report, where only 1 of 16 fatalities was due to MRSA, 35 more cases of fatal PVL-associated MRSA pneumonia have been reported [4,10–13,15–23,25–34,37,39,40].

In addition to these published cases, during 2005–2007 we have seen five cases of necrotising pneumonia: four due to unrelated strains of methicillin-sensitive PVL-producing *S. aureus* and one community-acquired (or community-associated) (CA)-MRSA strain. Three patients aged 18, 23 and 30 years died, two in the Emergency Department within a short time of admission with purpura fulminans. The other patient died after 5 days of what appeared initially to be successful therapy, with eventual failure of ventilation due to grossly necrotic and liquefied lungs revealed at post mortem.

4. MRSA producing PVL: CA-MRSA

CA-MRSA can be defined as an isolate obtained from an outpatient or from an inpatient during the first 48 h of hospitalisation in the absence of identified risk factors for acquiring MRSA. CA-MRSA usually carries a gene encoding PVL and staphylococcal chromosomal cassette (SCC) *mec* element types IV or V. Both code for methicillin resistance, but isolates usually lack other antibiotic resistance genes. Generally, recognition of the PVL status of an organism by its phenotype or antibiogram is unreliable, although there are certain recognised clonally associated sensitivity patterns associated with different geographical locations. Most USA300 strains of MRSA are resistant only to β-lactams and macrolides, but recently mupirocin, tetracycline, clindamycin and fluoroquinolone resistance has been reported [59,60]. Clindamycin resistance is particularly prevalent in the CA-MRSA strains in Taiwan [39].

Historically, compared with hospital strains, CA-MRSA strains were recognisable by their comparatively increased sensitivity to antibiotics such as clindamycin, ciprofloxacin and co-trimoxazole. Latterly, however, CA-MRSA strains are acquiring more resistance factors and recently in Italy a case of CA-MRSA pneumonia with lessened vancomycin susceptibility was reported [58].

CA-MRSA strains are more likely to contain other toxin-producing genes, although coexistence with TSST-10-producing genes is uncommon. Some PVL-associated clones of MRSA are particularly noteworthy, namely USA300 and USA400, the former seemingly more 'fit' for evolutionary superiority and survival. The 'spreadability' of staphylococci expressing PVL appears to be enhanced, possibly by better adherence to damaged skin and particularly airways [61].

5. Pathogenesis of PVL-associated staphylococcal pneumonia

Haematogenous spread from a concurrent skin focus is uncommon, although some patients may have a history of skin lesions or contact with a person with infected skin lesions [12,16,17,20,27,32,34,35,41,49,51,53,54,56,57].

Relatively few patients developing necrotising pneumonia have a previous history of skin sepsis themselves [8,12,17,32,37,51,56], although some have close household contacts with skin and soft-tissue infections [34,35,49,57]. A father and daughter were both admitted with necrotising pneumonia; only the daughter survived [35]. Furunculosis pre-dated pneumonia in a 52-year-old man [17]. Incision and drainage of a thigh abscess was followed 2 weeks later by fulminant pneumonia in a 28-year-old man [12], and a 12-year-old boy admitted with a 2 cm papule on the lip died of MRSA pneumonia [32]. The time between skin sepsis and pulmonary infection may be as long as 6 months [37].

PVL-producing *S. aureus* has a propensity to attach to exposed collagen, with a particular affinity for basement membranes exposed by viral denuding of the epithelium, and hence is particularly attracted to damaged bronchial mucosa. Once established, rapid bacterial multiplication follows, with sheets of staphylococci producing other cytotoxins such as haemolysins that further promote tissue damage and bacterial spread [61]. A quorum-sensing system, accessory gene regulator, induces gene expression at the high cell density typical of PVL-associated staphylococcal infection, probably responsible for expression of protease and the particularly cytotoxic alpha toxins. Leukocidin protects the staphylococci by destroying approaching polymorphs, and the synergy between the various necrotising staphylococcal toxins, particularly α-haemolysins [62], results in a necrotising vasculitis with massive areas of infarction and haemorrhage.

Post mortem changes vary from massive acute intra-alveolar and interstitial haemorrhages and infarction through to completely necrotic lung with liquefaction in patients who take longer to die. On microscopy, sheets of Gram-positive cocci, often with a paucity of neutrophils, are characteristic. Whilst the proportion of damage actually due to PVL has

been disputed in the literature [63,64], PVL presence overall increases the risk of dying. In a series of patients with MRSA pneumonia, 100% ($n = 5$) of PVL-positive patients died compared with 47% of PVL-negatives, a relatively increased risk of 1.56 [30].

6. Clinical presentation and diagnosis of PVL-associated staphylococcal pneumonia

Early clinical suspicion of the presence of PVL in a respiratory infection is difficult, and subtle signs may be easily missed. The presentation can be protean, with flu-like illness that may genuinely be due to influenza, or staphylococcal bacteraemia. Skin and soft-tissue infection or thrombosis may precede clinical signs of pneumonia.

It is difficult to miss the 'full-blown' classical syndrome of haemoptysis, leukopenia, a respiratory rate >40/min and tachycardia described by Gillet et al. [4].

Dyspnoea out of proportion to clinical findings of pneumonia as well as a dusky cyanosis of nail beds and lips with dyspnoea has traditionally been associated with staphylococcal pneumonia [2]. With PVL-associated pneumonia, a rapidly progressive pneumonia evolves into acute respiratory distress syndrome [4]. On examination, fever >39 °C, a respiratory rate >40/min and tachycardia >140 beats/min (bpm) make the diagnosis likely. Significant haemoptysis and hypotension clinch the diagnosis [4].

However, depending on the time since infection and any effective intercurrent antibiotic therapy, patients may exhibit few or none of these symptoms. Early diagnosis is made more difficult especially in previously fit young people, who have physiological resilience and coping mechanisms to deal with serious infection early on and who may present with subtle unremarkable chest radiography changes and a seemingly unremarkable initial blood count. Whilst pyrexia, myalgia and chills suggest a non-specific or viral illness, the clinician should remember that these are features of other staphylococcal toxin production. Four patients who later represented with severe pneumonia were originally discharged from healthcare facilities with symptomatic treatment, only to return later in extremis [11,12,57]. In such cases, perhaps only a raised C-reactive protein (CRP) may alert the clinician that the diagnosis may be more than a simple exacerbation of asthma or bronchitis.

When assessing the severity of pneumonia in young people, age-dependent scoring systems such as the CURB65 score are misleading and should be avoided. The presence of any blood in sputum should alert the clinician to the possibility of PVL production and pneumonia despite taking antibiotics effective against commoner pathogens such as pneumococci but not against staphylococci may be another clue [13,17,22,50].

In practice, adequate diagnostic virology is rarely done and, because staphylococcal bacteraemia produces many symptoms of viral infection, the true incidence of preceding viral illness is unknown. Positive blood cultures are comparatively unusual in PVL-associated pneumonia compared with deeper foci such as osteomyelitis [65].

Pleural effusions have always been common in staphylococcal pneumonia, especially in children, and are equally prominent in PVL-associated disease [2,32,43,58]. The whole spectrum of complications can be seen in clinical practice, ranging from classical lobar pneumonia and pneumatoceles to empyema and septic emboli, in a series of patients from Texas [20].

If the characteristic multilobar lung infiltrates are present, together with skin and soft-tissue infection or osteomyelitis, staphylococcal infection is likely [66]. For those who survive, resolution of treated infection may take weeks or months. Fatal massive haemorrhage may supervene, even weeks later [51].

7. Laboratory investigations

Marked leukopenia [4] and recently lymphopenia has been noted in several cases [25]. When the patient is first seen, the white blood count may, however, appear within the normal range (presumably reflecting consumption and destruction of the initial massive polymorph response to infection), only later to be followed by profound leukopenia. Why many patients do not become leukopenic is unknown.

In contrast to pure viral infections, very high CRP levels (≥300 g/L) are usually present [28,40,56–58] reflecting the gross tissue destruction, thrombosis and bacterial sepsis.

Toxic shock and possibly myositis should be suspected if the creatine kinase is significantly raised [11,25,27]. Levels from 639 IU/L [25] to >34 000 IU/L [27] have been reported.

Gram staining of the sputum should reveal sheets of staphylococci, with a paucity of neutrophils with some red cells. Blood cultures may be positive but are less commonly positive with pneumonia than with other systemic infections.

In summary, based largely on features noted by Gillet et al. [4], a classical presentation of PVL-associated pneumonia would involve a previously fit young patient presenting with an influenza-like illness (pyrexia, myalgia, chills) ± diarrhoea and vomiting due to other staphylococcal toxin production ± toxic shock [27]:

- fever >39 °C;
- tachycardia >140 bpm;
- haemoptysis [4,13,25,27,41];
- hypotension;
- marked leukopenia [11,13,18,21,25,48] (but be aware may be 'normal' early on);
- multilobar infiltrates on chest radiography, usually accompanied by effusions and often cavitation;
- very high CRP level (often ≥200–350 g/L) not found in viral infection [10,40,53,56,57]; and
- Gram film of sputum reveals sheets of staphylococci.

8. Radiological investigations

Fifty years ago, the commonest presenting radiological appearances of staphylococcal pneumonia were several small rounded areas of consolidation that usually cavitate within 96 h, resulting in resolution, pneumatocele, fistulae, empyema or necrosis [2] Coalescence of small cavities was almost pathognomonic of staphylococcal pneumonia [2]. Then, the radiograph could be completely normal or with minimal findings in the first 24–48 h [2], only later developing multiple lobe involvement of scattered pneumonia areas in both lung fields. If the entire hemithorax was involved in infant pneumonia, almost invariably staphylococci were the cause [2].

Multilobular alveolar infiltrates are still usual for PVL-associated staphylococcal pneumonia and, unlike hospital-acquired MRSA pneumonia, more frequently cavitate, whilst effusions commonly develop [4].

However, in practice, acute infections may initially produce few if any chest radiograph changes, leading clinicians to misdiagnose infections as simple exacerbations of bronchitis or asthma [12,37,57]. The development of radiological changes is thereafter very rapid, reminiscent of 'old-fashioned' staphylococcal pneumonia. Single or multiple opacities <3 cm diameter are suggestive of staphylococcal infection. Overall, the incidence of complicated pneumonia is far higher than with non PVL-producing staphylococcal pneumonia.

Cavitation is seen on serial radiographs and may be apparent earlier with ultrasound. However, computed tomography (CT) scanning or magnetic resonance imaging allows the best evaluation of the ongoing pathology, particularly with cystic changes [50,53]. The classical multilobular infiltrates and diffuse multilobar opacities followed by cavity formation are best confirmed with CT and may develop after only a few days [27,52,58].

9. Treatment of PVL-associated staphylococcal pneumonia

The initial management of necrotising pneumonia is supportive, with intensive care, ventilation and aggressive antibiotics. In addition to routine infection control precautions, it seems sensible that masks should be used during intubations and physiotherapy where exposure to respiratory secretions may occur. Closed tracheal suction should be used to prevent secondary cases due to respiratory spread [14].

Unfortunately, 'even appropriate antibiotics have a limited capacity to alter the outcome of severe infections' [67]. Overall, little seems to have changed from the 'second wave' of the 1919 influenza outbreak in Fort Jackson, when hundreds of troops were dying, very probably of PVL-related necrotising pneumonia. Clinicians then commented 'the treatment of *Staph. aureus* infection of the lung is extremely ineffectual' [67].

With an expected mortality approaching 75% [4], it is imperative to give the correct antimicrobials as soon as possible. β-Lactams can no longer be relied upon to cover staphylococci. Since α-haemolysin is a major contributor to the necrotising process [62] and since *hla* expression is strongly induced by β-lactams [68], perhaps they are best avoided altogether. Furthermore, since nafcillin upregulates PVL toxin and α-haemolysin production [68], there is a possibility that flucloxacillin may increase PVL production in vivo as it does in vitro. Whilst the addition of flucloxacillin for bactericidal action to linezolid or clindamycin may seem inherently sensible, there is the possibility that the low concentrations of flucloxacillin achievable in vivo in the poorly perfused necrotic tissue may further augment PVL toxin production [68].

In 1999, unsuspected MRSA as a cause of pneumonia in four Minnesotan children treated initially with empirical cephalosporins explains why all died. The general increase of MRSA prevalence worldwide and the rapid emergence and spread of the USA300 CA-MRSA clone in particular necessitate consideration of empirical cover for MRSA in all cases of suspected staphylococcal pneumonia. Using antimicrobials effective against MRSA that also decrease exotoxin production may be optimal therapy. Conventional doses of vancomycin produce inadequate lung concentrations for MRSA in many patients [68]. Vancomycin has no effect on exotoxin formation, and even with high trough serum levels breakthrough continuous bacteraemia has been reported days into therapy [50].

Although reportedly successful as sole therapy in a 46-year-old Latvian with pneumonia secondary to an infected cut [51], vancomycin as sole therapy was successful in only one other case reported to date [41]. Vancomycin appears not to be very successful in vitro, either with rifampicin where antagonism may be present [69], or in vivo [50]. Even with high trough levels of between 15 and 20 mg/dL [69], patients remained bacteraemic with positive bronchial lavage 3 days into therapy. Isolates with increased minimum inhibitory concentrations (MICs) to vancomycin on presentation have been described [56,58,70].

It is an enigma why some patients survive such devastating illness despite never receiving 'effective' antibiotics [71], whilst 14/25 cases reviewed died despite having received timely appropriate therapy [72].

Whilst most CA-MRSA strains remain sensitive to co-trimoxazole, there is only one report of its use in severe pneumonia to date. The 26-year-old male patient survived [37].

Various combinations of vancomycin, clindamycin, linezolid, rifampicin and co-trimoxazole have been used in differing doses and combinations in PVL-associated pneumonia cases, with varying degrees of success [4,27,50,71,73].

Subinhibitory concentrations of clindamycin, linezolid and fusidic acid all induce a concentration-dependent decrease of PVL levels, whereas with low concentrations of oxacillin the level of PVL increases up to three-fold [74].

Clindamycin and linezolid have the advantage of switching off toxin production [75], and clindamycin decreases TSST-1 production by 95% in stationary phase cultures [76]. Linezolid is active against MRSA, although the activity of clindamycin is variable. Clindamycin stops alpha toxin production by translational inhibition, particularly the normal peak of alpha toxin production occurring during the late exponential phase of growth [77]. Clindamycin and linezolid both markedly suppress PVL production as staphylococci approach stationary phase, with none detectable up to 12 h later [68].

Staphylococcus aureus isolates resistant to erythromycin but apparently clindamycin-sensitive must be 'D tested' to exclude inducible clindamycin resistance. There are striking differences in the rates of inducible clindamycin resistance worldwide, again related to clonality of the predominant strains, with a fall in resistance sometimes occurring due to a clonal shift [78]. Of the newer antimicrobial agents active against MRSA pneumonia, linezolid, appears to be the most promising, especially in CA-MRSA strains. Treatment successes have been reported by several authors, often as second-line therapy in those failing other treatments [38,44,49,50,52,58], and we have found it successful in three patients with necrotising pneumonia to date.

Three of four patients with necrotising pneumonia clinically failing vancomycin therapy responded to a change to linezolid and rifampicin [50]. A PVL-positive USA300 MRSA strain causing necrotising pneumonia in a 16-year-old Italian boy, with a vancomycin MIC of 2–4 g/L, responded to a unique combination of linezolid, teicoplanin and rifampicin, but the patient was hospitalised for 6 weeks [58].

It is unfortunate that whilst daptomycin is extremely rapidly bactericidal in vitro, inactivation by surfactant limits its usage to non-pneumonic infections [79]. For primarily haematogenous pneumonia with septic emboli there may theoretically be a role for daptomycin in combination with other antimicrobials, since it may lessen the release of toxins and inflammatory mediators.

Moxifloxacin is far superior to ciprofloxacin in treating CA-MRSA strains [80] but worries about encouraging MRSA resistance prevent its more widespread use. Although tigecycline was active against 89 of 91 strains of CA-MRSA in phase 3 trials [81], no one has reported clinical usage of a tetracycline in PVL-associated pneumonia to date.

When successful, the duration of therapy for complicated non-PVL-producing staphylococcal pneumonia may be prolonged for weeks [82]. One patient with bronchiectasis and PVL-associated staphylococcal pneumonia was reportedly still on therapy many months later [49].

10. Adjunctive therapy for PVL-associated infections

Whilst anecdotal reports suggest a possible role for activated protein C in severe staphylococcal sepsis [38], we have not used it as there is likely to be active haemorrhage ongoing even in the early stages of infection.

The later any therapy is started, the more toxins are already present in the lung substance, with concomitant tissue damage, and toxins already produced need to be neutralised. Intravenous immunoglobulin (IVIg) neutralises PVL pore formation and the cytopathic effect of PVL in vitro, with inhibition being concentration-dependent [83], and has been used in six patients with PVL-associated pneumonia reported to date [10,12,38,44,56]. The optimal dosage of IVIg is uncertain; that recommended for streptococcal toxic syndrome is 2 g/kg [84,85] repeated at 48 h if there is still evidence of sepsis or failure to respond. In Exeter, we have used the same dosage successfully for systemic PVL infections. Although neutralisation of staphylococcal toxic shock syndrome toxins may justify a higher dosage of IVIg [84], no one has yet reported using more than 2 g/kg in PVL-associated disease. The combination of linezolid and IVIg was particularly effective in a boy with septic arthritis and pneumonia, who was discharged to the ward on Day 5 [44].

Granulocyte colony-stimulating factor has been used in two neutropenic patients (with necrotising pneumonia) [38]. Extracorporeal membrane oxygenation (ECMO) has been largely unsuccessful [10,11,34,37] for patients with failure of ventilation or unresponsive purpura fulminans, except for one case of necrotising pneumonia [38]. ECMO may be useful as an interim measure where lung transplantation may be an option.

Although of theoretical benefit in very early sepsis, once active haemorrhage has occurred activated protein C should not be used, hence it has no role in PVL pneumonia. Protein C has been used in very few patients; in one patient with toxic shock syndrome and PVL pneumonia who survived [27], and in another patient in a 'brief trial' [17].

11. Conclusion

With few guidelines available and no double-blind randomised controlled trials ever likely to be conducted, we have adopted a pragmatic local approach to the therapy of PVL-associated pneumonia. With our experience of successfully treating other necrotising infections (group A streptococcal pneumonia, fasciitis and necrobacillosis), we use large doses of antibiotics primarily aimed at switching off exotoxin production. We avoid β-lactams even if the isolate is proven to be methicillin-sensitive and we do not use vancomycin.

Combining clindamycin with linezolid is synergistic in vitro [86] and is our preferred initial therapy pending antimicrobial sensitivities and exclusion of inducible clindamycin resistance. We use the same dosages of antimicrobials for PVL-associated disease as we use in necrotising fasciitis [87], namely 1.2–1.8 g intravenous clindamycin 6-hourly and intravenous linezolid 600 mg 12-hourly.

We have used IVIg 2 g/kg successfully in three patients. However, whilst circulating exotoxin is comparatively easy

to 'mop up' with IVIg, the inexorable necrotising activity of toxin already produced continues unimpeded, further aided by the poor penetration of agents into the necrotic lung tissue. Depressingly, we are now no further forward than nearly 50 years ago, when it was said that 'In certain of our pts we have achieved bacteriologically sterile cultures [blood serous fluids throat and sputum] yet coalescing of abscesses, perforation of the pleura and persistent fever has continued ... possibly the remaining toxin—e.g. the necrotoxin—remains active although the organism has been killed or is no longer reproducing' [2].

Inactivation of the toxins driving pulmonary necrosis must be the key to improved patient survival. We need to be able to remove toxins or inactivate them at an earlier stage. Potential areas for research include adjunctive nebulised immunoglobulin, which we have used very successfully in conjunction with IVIg in one case of necrotising pneumonia, and possibly glycerol monolaurate [88]. Until the toxins and inflammatory intermediaries responsible for the uncontrollable necrosis in these very sick patients can be neutralised earlier, the outlook will remain bleak.

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