

Panton-Valentine Leukocidin: A Review

Marina Morgan F.R.C.Path.
Venkata Meka, M.D.

Panton-Valentine leukocidin (PVL) is a bi-component, pore-forming exotoxin produced by some strains of *Staphylococcus aureus*. Also termed a synergohymenotropic toxin (i.e. acts on membranes through the synergistic activity of 2 non-associated secretory proteins, component S and component F) (9), PVL toxin components assemble into heptamers on the neutrophil membrane, resulting in lytic pores and membrane damage. Injection of purified PVL induces histamine release from human basophilic granulocytes, enzymes (such as β -glucuronidase and lysozyme), chemotactic factors (such as leukotriene B₄ and interleukin (IL-) 8), and oxygen metabolites from human neutrophilic granulocytes (5). Intradermal injection of purified PVL in rabbits causes severe inflammatory lesions with capillary dilation, chemotaxis, polymorphonuclear (PMN) infiltration, PMN karyorrhexis, and skin necrosis (17).

PVL production is encoded by two contiguous and cotranscribed genes, lukS-PV and lukF-PV, found in a prophage segment integrated in the *S. aureus* chromosome (9).

Traditionally some 2% of *S. aureus* produce PVL, however in certain groups in close contact with each other, such as military personnel, prisoners, and families of patients with infected skin lesions, skin to skin transmission is common resulting in a far higher carriage rates. Different PVL-positive *S. aureus* strains carry differing phage sequences, which can move into other strains methicillin and resistant, empowering them with PVL production genes (15). Many patients with PVL-positive necrotizing pneumonia have a preceding illness resembling influenza, with rigors pyrexia and myalgia. Expression of most *S. aureus* exoproteins (including toxins such as PVL and enzymes) is controlled by the quorum-sensing system, agr (accessory gene regulator), which induces their gene expression at high organism density and may be responsible for expression of protease and the particularly cytotoxic alpha toxins.

Controversy Regarding the Role of PVL in Necrotizing Infections

PVL is epidemiologically associated with necrotizing skin and soft tissue infections and necrotizing pneumonia, and experimentally established as a virulence factor in cutaneous infections in rabbits, although variable results occur in other animal models. PVL-associated disease seems generally to be associated with a more severe inflammatory response (1).

PVL production is especially associated with clones of community-associated Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) such as USA-300. CA-MRSA seems to be far more pathogenic than hospital associated strains, causing epidemics of skin and soft tissue infections (SSTI) in most parts of the world. However PVL may not be the

only virulence factor for necrotizing infection. In their mouse model, Voyich and colleagues reported striking differences between the ability of two different PVL producing MRSA strains to produce skin necrosis (15).

Furthermore, not all CA-MRSA produce PVL, and *in vitro* production of PVL does not necessarily correlate with severity of infection. However, USA-300 and related clones of CA-MRSA are increasingly associated with severe invasive infections including necrotizing pneumonia, pyomyositis, necrotizing fasciitis, ocular infections, Waterhouse-Friedrichsen syndrome (purpura fulminans) a particularly aggressive form of osteomyelitis associated with deep venous thromboses in children (1, 7) and recently endocarditis.

Whether PVL is merely a co-factor in the causation of necrotizing pneumonia is the subject of much debate (3). PVL genes were detected in 23/27 *S. aureus* strains causing primary community-acquired pneumonia, and all 14 deaths were in PVL-positive patients (9). Significant findings on autopsy included bilateral necrotizing hemorrhagic pneumonia, and histology showed necrotic lesions of the tracheal mucosa and alveolar septa with numerous clusters of Gram-positive cocci.

The epidemiological association of PVL-producing *S. aureus* strains from patients with necrotizing pneumonia suggested PVL was a major virulence factor (5). Labandeira Rey et al., using a laboratory strain of *S. aureus* in mice, confirmed PVL alone could cause lethal infection (8). Voyich et al (15) and Wardenburg et al (18) failed to establish the role of PVL as a virulence factor in mouse models of infection. Wardenburg et al. showed that *S. aureus* incapable of producing alpha hemolysin was completely incapable of causing pneumonia, whereas deletion of PVL genetic loci in infecting strains had no effect on mortality (18). Alpha hemolysin and peptides such as the phenol-soluble modulins (PSMs) are now mooted as likely virulence co-factors in these severe necrotizing infections (15, 16). Loss of PSM alpha reduced the virulence in a mouse model of staphylococcal bacteremia and abscess formation (16).

At present, PVL is at least a marker for the more pathogenic forms of staphylococci, and a highly-linked epidemiological marker for CA-MRSA, “the notion that PVL production is critical for disease... is widely accepted.” (15).

Epidemiology

The PVL gene attained added significance with the worldwide emergence of CAMRSA infections. CA-MRSA can be defined as an isolate obtained from an out-patient or inpatient during the first 48 hours of hospitalization in the absence of identified risk factors for acquiring MRSA. CA-MRSA usually carries a gene encoding for PVL and staphylococcal chromosomal cassette (SCC)mec element types IV or V, encoding for methicillin resistance. Originally CA-MRSA strains were easily identifiable being comparatively antibiotic sensitive as compared with hospital strains, but now reports of resistance are increasing, even some exhibiting lessened vancomycin susceptibility.

Although some patients with CA-MRSA cutaneous infection have no MRSA-related risk factors, those at increased risk of infection are the newborns, men who have sex with men, prisoners, military and sporting personnel, and certain native ethnic populations.

Clinical Manifestations of PVL-Associated Diseases

With an apparent predilection for cutaneous infections, PVL production was initially linked to furuncles, cutaneous abscesses, and necrotic skin infections (13). Subsequently PVL genes were reported in *S. aureus* strains causing primary skin infections and primary community-acquired pneumonia (unlike strains causing post-traumatic secondary skin infections or hospital-acquired pneumonia). A common manifestation of PVL-related infection is a localized necrotizing skin lesion resembling a *Loxosceles* spider bite, and reported particularly with CA-MRSA. Endocarditis, purpura fulminans (reminiscent of meningococcal septicaemia) and necrotizing fasciitis are uncommon presentations.

Gillet et al. compared the characteristics of sixteen cases of community-acquired pneumonia due to PVL-positive *S. aureus* strains to 36 cases of PVL-negative *S. aureus* pneumonia. The PVL-positive patients were much younger (median 15 years) when compared to PVL-negative patients (median age 70 years). PVL-positive patients were significantly more likely to have temperature $>39^{\circ}\text{C}$, tachycardia above 140 beats per minute, hemoptysis, pleural effusion, and leukopenia. The mortality 48 hours after admission was significantly higher for the PVL-positive patients as compared to the PVL-negative patients, and overall 12/16 (75%) PVL positive patients died. Histology from three autopsies showed extensive necrotic ulceration of the trachea and bronchial mucosa with massive hemorrhagic necrosis of the interalveolar septa (5).

An analysis of 50 French patients with PVL pneumonias reported a mortality of 56%, death being due to refractory shock or respiratory failure. A history of furunculosis was found in 17.6%, whilst 24% had pre-existing staphylococcal infections, including abscesses. Some 67.3% reported a flu-like illness, but only nine patients actually had virology samples taken for investigation. Of the nine, 4 proved positive for influenza A, and one Cytomegalovirus. To complicate matters, the preceding flu-like illness, with temperatures, rigors and myalgia, may also be due to symptoms of staphylococcal bacteremia, especially in those necrotizing pneumonia that are hematogenous in origin. Hemoptysis, uncommon in children, was reported in 44% of cases (6).

A primary viral lung infection, with desquamation of ciliated and secretory cells, exposes the collagen, allowing bacterial adhesion to basal epithelial cells (9) and a secondary aspiration staphylococcal pneumonia. PVL-producing *S. aureus* have a higher affinity for basement membranes (2). Once established, rapid bacterial multiplication and further production of exotoxins such as hemolysins promote tissue damage and further bacterial spread (2). High severity of illness scores, airway bleeding, erythroderma and leucopenia are significantly associated with death (6). Pleural effusion present at admission and a personal history of furunculosis were associated with survival (6).

Management of Cutaneous PVL Infection

Whether CA-MRSA or methicillin sensitive strains, incision and drainage of boils and abscesses smaller than 5cm diameter may suffice, otherwise oral antimicrobials such as co-trimoxazole or clindamycin are commonly used. There is debate about the usefulness of

attempting decolonization of symptomatic PVL-positive staphylococcal carriers, for CA-MRSA and PVL-positive MSSA, with recommendations differing between countries. Education on the importance of hygiene measures, such as hand washing, avoiding sharing baths and covering open wounds, is a mainstay of prevention of spread of infection.

Management of Necrotizing Pneumonia

Necrotizing pneumonia, with a mortality of 56% - 63% (6,10) is very more difficult to treat. The classical clinical symptoms and multilobar shadowing may not be apparent in early presentations and the diagnosis of “query Viral infection” is often made. Later presentations are more obvious, with leucopenia and hemoptysis being particularly gloomy prognostic indicators. Treatment with conventional cell-wall active agents such as vancomycin or beta-lactams such as oxacillin has drawbacks, with no effect on toxin production. Conventional vancomycin doses often produce inadequate lung concentrations for killing MRSA, will not affect toxin formation, and even with high trough serum levels, breakthrough continuous bacteremia has been reported days into therapy (11). Conversely, antibiotics that directly inhibit protein/exotoxin synthesis, such as clindamycin and linezolid, markedly suppress PVL-production in staphylococci (4) whereas nafcillin up-regulates both PVL toxin and alpha hemolysin production (4). Even when the bacteria are dead, PVL and alpha toxins already present in tissues may continue to cause necrosis (15).

None of the variety of antimicrobial combinations reported has demonstrated clear superiority, although combinations involving linezolid and rifampicin together with intravenous immunoglobulin have been successful and merit further consideration (15).

With death due to continuing lung necrosis and failure of oxygenation, a variety of adjunctive therapies have been tried in desperation with some success. These include IVIg, extra corporeal membranous oxygenation (ECMO) and eventual transplantation (15). Commercial IVIg preparations contain antibodies against PVL that in vitro neutralize leukocyte pore formation and the cytopathic effect of recombinant PVL and *S. aureus* culture supernatants (4). Intravenous immunoglobulin seemed effective in 2 patients who, given their condition, were expected to die (13). The clinical dosage of immunoglobulin reported is 2gm/kg (10).

A plethora of educational initiatives and guidelines for prevention of spread of infection with PVL producing MSSA and CA-MRSA has resulted in better public awareness. However, many patients still re-present time and again with recurrent SSTI before the diagnosis is considered, and eradication of carriage, where attempted can be unsuccessful for several cycles. Whilst the more severe manifestations of PVL-associated disease are still relatively uncommon, the epidemic spread of some of the CA-MRSA clones is worrying. Whether we will see a concomitant increase in PVL associated disease remains to be seen.

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