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Clinical and clinical laboratory aspects of nocardial infection

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INTRODUCTION

It is nearly 100 years since Nocard described bovine farcy, a granulomatous disease of cattle characterized by abscesses, sinus tracts and pulmonary disease (Nocard, 1888). This was the first disease due to *Nocardia* species described. Since that time our knowledge of the role of the nocardiae in human veterinary diseases, of the epidemiology of these diseases, and their diagnosis and treatment, has enlarged rapidly. This paper attempts to give an overview of our current understanding of these diseases.

EPIDEMIOLOGY

A collation of epidemiologic information (Beaman *et al.* 1976) indicates a minimum of 500–1000 human cases/year in the USA alone, although recent clinical reports from many centres all indicate that the incidence of infection is rising. This may be due in part to improvements in diagnosis, and to improved survival of patients in the immunosuppressed state because of more agressive therapy of malignancies and more vigorous immunosuppressive therapy of rejection of transplants, and to improvements in therapy of other opportunistic infections (leaving the patients vulnerable to the possibility of later infection with nocardiae). There seems no geographic predilection, with the exception that mycetomas due to *Nocardia* species occur predominantly in tropical areas. In Europe and North America, about 85% of the infections seen are pulmonary and/or systemic, with estimates that 75–79% of cases will have some pulmonary involvement and 45% systemic dissemination. Between 44 and 85% of cases are seen as opportunistic infections in immunocompromised hosts (Young *et al.* 1971) in various series, although only 25% of cutaneous infections occur in this group. All but 9–19% of human nocardial infections in various species are due to *N. asteroides*; most of the primary cutaneous infections are due to *N. brasiliensis* and, conversely, the infections due to *N. brasiliensis* are mostly cutaneous infections. Overall, infections in males predominate over females 2–3 : 1.

The instances of primary cutaneous inoculation and the predominance of pulmonary infections suggest the principal source of infections is from a reservoir of organisms in the soil and thence via an airborne route. However, episodes of clustering of paediatric cases (Cox & Hughes, 1975) and of nosocomial cases (Rosett & Hodges, 1978) have suggested that patient-to-patient transmission might occur directly or indirectly. A recent outbreak of *N. asteroides* infections in a renal unit

in a London hospital provided the strongest argument for contagion to date (Houang *et al.* 1980; Lovett *et al.* 1981; Stevens *et al.* 1981).

In this outbreak, in a 9 month period, seven cases were proven by culture and two detected serologically. This led to closure and cleaning and fumigation of the unit. Most of the cases had pulmonary involvement. Serological investigations of patients and staff did not suggest the presence of subclinical cases. Epidemiologic investigations yielded cultures of *N. asteroides* from air and dust inside the unit and elsewhere in the hospital. Characterization of the isolates by biochemical, metabolic, physical and immunologic methods indicated that those from patients and those from the unit environment were identical, whereas some from outside the unit could be differentiated from these (Stevens *et al.* 1981). The epidemic strain had an antigen, Type III (see discussion below), which is not the most common type involved in human cases in the United Kingdom (nor in the United States; Pier & Fichtner, 1981). The methods of characterization used successfully in this study could be usefully employed in further studies of nocardical epidemiology. Although current hospital guidelines don't recommend respiratory isolation of cases of pulmonary nocardiosis, this should be reconsidered in view of this experience, particularly if there are immunocompromised hosts in the environment.

DIAGNOSIS

Smear and culture

Nocardiae appear in clinical specimens as Gram positive slender branched filaments (Buechner *et al.* 1973; Palmer, Harvey & Wheeler, 1974). If such structures are seen, the principal differential diagnosis is with disease due to actinomycetes. These can be later differentiated by the fact that nocardiae grow aerobically and actinomycetes anaerobically, but actinomycetes can grow in a microaerophilic environment, and with high inocula give rise to small colonies on aerobic plates. They may also be differentiated on the smear by testing for acid-fast staining properties. Actinomycetes are not acid-fast, whereas nocardiae have this property. However, their acid-fastness is relative compared to that of mycobacteria; to demonstrate this with the Ziehl–Neelsen methods requires 5–20 s of decolorizing, as opposed to the more than a minute commonly used to demonstrate mycobacteria. It should also be pointed out that this property of acid-fastness applies to nocardiae obtained from tissue, a property not shared by nocardiae taken from culture plates.

The differential diagnosis with actinomycetes is also a consideration when so-called 'sulphur granules' (actually masses of matted organisms that appear yellow without staining) are noted in suppurative lesions. Although these have been classically associated with actinomycetes, nocardiae can produce these too, as do fungi and other bacteria (Graybill & Silverman, 1979).

While the filaments are easily seen with the Gram stain, they are not stained by the hematoxylin and eosin methods. A further source of confusion is that the filaments may break up in the tissue or in processing, giving rise to bacillary forms that are most reminiscent of mycobacteria. The organisms will, like fungi, be detected with silver stains such as the Gomori-methenamine-silver stain, but will not be detected with the periodic acid Schiff stain (as will fungi).

Nocardiae will grow from clinical specimens on several media at 37 °C, as dendritic colonies. In general, blood agar plate are superior to Lowenstein–Jensen medium (used primarily for mycobacteria), which in turn has a better yield than Sabouraud's medium used to culture fungi. However, Sabouraud's is superior to media utilized in the clinical laboratory which contain antibiotics, and the yield is even lower when specimens are digested first as for culture for mycobacteria. The yield is markedly improved by a CO_2 atmosphere. Specimens which are contaminated by normal flora or other pathogens may be a problem; incubating cultures at 40–50 °C will generally reduce this problem, while nocardiae can survive. Isolation from the blood in cases of nocardemia seems optimal using fungal media.

It is common that nocardiae are detected in clinical specimens in the mycology laboratory or mycobacteriology laboratory, because bacteriology laboratories do not usually hold their plates long enough. These organisms may grow in a few days, but can take up to 4 weeks to form colonies. If the clinician suspects the possibility of nocardial infection, he should request that the culture plates be held for this duration.

The organism is almost never grown from cerebrospinal fluid in instances of central nervous infection. Finally, with regard to culture for diagnosis, gastric aspirates are often not helpful because nocardiae can be found in several foods.

This emphasis on diagnosis is worthwhile, because several series have indicated that the delay between symptoms and diagnosis can average 12 weeks.

Serology

Because of the delay in diagnosis, a serological diagnostic technique would be useful. Nocardiae possess several antigens. There are polysaccharides, principally arabinomannan and arabinogalactan, which are shared with mycobacteria, corynebacteria, and rhodochrous species (Ridell, 1977; Ferguson *et al.* 1978). Ribosomal antigens cross-react with ribosomal antigens of mycobacteria and corynebacteria (Laub, Delville & Cocito, 1978). Despite these complexities, Pier and his colleagues were able to use the complex mixture of extracellular antigens produced in culture filtrates to define four prototype antigens (Pier & Keeler, 1965; Pier & Fichtner, 1971). Generally, one of these is produced per isolate, though occasionally two or three may be present. Types I–III are found in *N. asteroides*, type IV was also seen in other nocardiae. These studies enable typing of isolates, and this contributed to the laboratory definition of the London outbreak described above (Stevens *et al.* 1981).

These antigen mixtures were also used by us to develop a complement–fixation test to diagnose human nocardiosis (Shainhouse, Pier & Stevens, 1978). This test utilizes rabbit antisera against the extracellular antigens as the reference antiserum, is performed using microtiter methods, and uses overnight binding at 4 °C. The antigen is produced in neopeptone dialysate medium which is grown for 2 weeks at 37 °C on a shaker, and the medium is then filtered and sterilized with merthiolate. We found a titre of $\geqslant 4$ to be a useful cutoff. With this method, the test was 81 % sensitive in detecting nocardiosis using patient sera, and the rate was similar (82 %) if only immunocompromised hosts were considered. Positive reactions were detected in cases of pulmonary and of disseminated disease, and in fatal cases. False

positive reactions are a problem in patients with tuberculosis, and were noted also with leprosy sera, but not in patients with other infections. Titres are generally low, rarely exceeding 16.

Experience with this serological technique in the London outbreak (Stevens *et al.* 1981) indicated its utility in a clinical setting if used early in the clinical course, and with repeated testing at intervals. It appears that the antibody disappears with appropriate therapy, and so the test could be used to follow the course of an illness.

More recent studies in our laboratory have begun to define the antigens in nocardiae that are immuno-dominant, i.e., recognized by patient sera (Sugar *et al.* to be published). This plan of attack may improve further the utility of the test.

Others have reported experiences with different methodologies. A whole cell antigen (Humphreys, Crowder & White, 1975) has been used in a precipitin test, and 45% of cases studied were positive (22% in immunocompromised hosts). Cross-reactions with tuberculosis and leprosy patients were noted. The test was positive early in the illness in some instances, and detected one case in the incubation period (as we also have found with our complement–fixation test). A cytoplasmic antigen has been used in counter-immunoelectrophoresis, and found to be specific but not sensitive.

A micro-immunodiffusion method (Blumer & Kaufman, 1979) has been used to test results with a filtrate antigen and a homogenate antigen, and demonstrated them to be comparable. The use of reactions of identity was more specific and predictive than the presence of any band, but less sensitive. Patients with tuberculosis and actinomycotic infections gave false positive results. With the use of both antigens, the test was 47% sensitive (32% in immunocompromised hosts), with a 6% false positive rate (mostly due to tuberculosis).

CLINICAL PICTURE

Some of the clinical forms of nocardial infection have been mentioned in the section on epidemiology. The clinical picture is usually that of a suppurative process without 'tubercles' (caseating granulomas) (Louria, 1967; Idriss, Cunningham & Wilfert, 1975; Frazier, Rosenow & Roberts, 1975; Folb *et al.* 1976; Curry, 1980). Nocardiae may rarely be a saprophyte on the skin or in the upper respiratory tract, but is not part of our normal flora. In the presence of disease, isolation of nocardiae should always be regarded seriously. The most common exception to this is the predilection of the organism to grow saprophytically in old tuberculous pulmonary cavities.

Certain patients are predisposed to nocardial infection. The association with immunocompromised hosts in general has already been emphasized. There is a particular association with chronic granulomatous disease of childhood, and also with diabetes mellitus, and pre-existing pulmonary disease, particularly chronic obstructive lung disease and alveolar proteinosis.

As mentioned, pulmonary infection is most common (Baum *et al.* 1970). Abscesses are characteristic, and a presentation with chronic suppurative pneumonia and bronchitis is very common. However, necrotizing pneumonia, cavities, nodules, infiltrates or a miliary picture can be seen. In Europe and North America, where mycetomata are less common, infection of the central nervous system would

be the next most common presentation (Utz, 1977). This has been reported in series of patients with nocardiosis in 23–44 %. This almost always takes the form of a cerebral abscess.

Primary cutaneous infections have already been referred to, but skin and/or soft tissue infection may also occur consequent to dissemination. In disseminated disease, involvement of the liver, kidney, bones, lymph nodes, eye, ear, pharynx or pericardium has been recorded in several instances, as has stomatitis and endocarditis.

Observations on prognosis (Presant, Wiernick & Serpick, 1973) have indicated mortality is associated particularly with acute disease, symptoms less than 3 weeks, concomitant immunosuppressive therapy or Cushing's disease (with high endogenous steroid levels), and dissemination involving 2 or more non-contiguous organs. Others (Geiseler & Andersen, 1979) have suggested patients with immunosuppression who have only lung involvement and are treated early can do well, but that brain involvement is also significantly associated with mortality.

VETERINARY

Nocardiosis is also a problem of domestic animals. Most of these infections are due to *N. asteroides*, as is the case in man. Epizootics in herds of dairy animals and in kennels have been noted. In cattle, the infection characteristically produces mastitis (Pier & Enright, 1962). Sinus tracts and lymphangitis are common, but granules are not seen. In dogs, young animals are usually affected, commonly with a pulmonary infection and following viral distemper.

A skin test, which does not cross-react with mycobacterial infection, indicated in surveys that about 1 % of normal cows in the USA have been infected with nocardiae at some time (Pier, Thurston & Larsen, 1968).

TREATMENT

Most reports of *in vitro* susceptibility testing of isolates and clinical reports of responses to therapy deal with *N. asteroides* infections (Dalovisio & Pankey, 1978). There are no reports of comparative clinical trials of therapeutic regimens, so many different therapeutic approaches have been justified by their proponents. Furthermore, the relevance of *in vitro* testing to outcome is unclear, and is subject to many variables, such as inoculum size, etc.

Most clinical experience has been with responses to the sulpha drugs, at 4–8 grams/day. In animal models (Scholer, 1968), many of this class of drugs are active, although on a mg-for-mg basis sulphamethoxazole is less potent, and sulphanilamide is particularly weak. Othe regimens for which there is enthusiasm in the clinical literature include erythromycin, ampicillin, the combination of erythromycin plus ampicillin, cycloserine, or tetracyclines, particularly minocycline. Amikacin and the sulphones have been shown to be active in animal models (Strauss, Kligman & Pillsbury, 1951; Wallace *et al.* 1979). Other drugs have been reported to be active *in vitro* in some laboratories (e.g., streptomycin, capreomycin), but clinical enthusiasm is as yet limited; clindamycin has probably received the most clinical attention of this group.

Of the newer drugs, a growing confidence in the combination of trimethoprim

and sulpha is noted (Wallace *et al.* 1982; Smego, Moeller & Gallis, 1983), though failures of therapy (Geisler *et al.* 1979) are also being recorded. *In vitro* studies (Bennett & Jennings, 1978) suggest that the ratio of trimethoprim to sulpha is very important in the inhibitory activity, and also that with some isolates the combination may be antagonistic. Higher trimethoprim to sulpha ratios than that provided by the commercially available fixed combination appear to be more inhibitory. If adding more trimethoprim to the available combination raises questions about possible hematologic toxicity, possibly folinic acid could be given to such patients, since this does not seem to antagonize the anti-nocardia activity of the two antimicrobials.

In vitro activity amongst the beta-lactam drugs has been particularly noted with cefamandole, cefotaxime, ceftriaxone, cefuroxime, cefmonoxime, and thienamycin, although other cephalosporin drugs may also be active against some isolates (Cynamon & Palmer, 1981; Gombert, 1982; Gutmann *et al.* 1983). We have found cefotaxime to be highly effective in an animal model (Sugar, Chahal & Stevens, 1983). Nocardiae commonly possess an inducible beta-lactamase, although the relationship of this to resistance to beta-lactam drugs is uncertain. This suggests the possibility that beta-lactamase inhibitors, such as clavulanic acid, may be useful clinically in combinations with beta-lactam drugs.

N. brasiliensis infections, such as mycetomas, are reportedly responsive to dapsone 100 mg b.i.d. for 2 years.

Recommendations for the duration of therapy of the deep nocardial infections more commonly seen in Europe and North America are also variable, and again, are unsupported by comparative data. One school of thought advises durations on the order of 2–4 months, or 6 weeks after clearing of disease. The other advises minimum durations of about a year, for they are impressed by the high per cent of failures and relapses reported with less treatment. Recommendations for duration of therapy of cutaneous infections are less variable; treatment until healing with a minimum of 2 weeks represents one approach, whereas others recommend 6–8 weeks treatment.

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