Clinical and Histopathological Features and a Unique Spectrum of Organisms Significantly Associated with Chronic Granulomatous Disease Osteomyelitis during Childhood


Servicio de Patología, Laboratorio de Microbiología, and Servicio de Inmunología, Hospital Nacional de Pediatría J. P. Garrahan, Buenos Aires, Argentina

With an incidence of ∼1 cases per 250,000 newborns, CGD is a rare disease characterized by recurrent life-threatening infections, as well as by dysregulated inflammatory mechanisms [1]. With an incidence of ∼1 cases per 250,000 newborns, CGD is a rare disease characterized by recurrent life-threatening infections, as well as by dysregulated inflammatory mechanisms [1]. Chronic granulomatous disease (CGD) is a genetically inherited rare disease characterized by recurrent life-threatening infections, as well as by dysregulated inflammatory mechanisms [1]. With an incidence of ∼1 cases per 250,000 newborns, CGD is caused by defects in nicotinamide adenine dinucleotide phosphate oxidase, the enzymatic complex responsible for respiratory burst and reactive oxygen species generation within the phagocytes [1, 2]. Anti-infective prophylaxis is the standard of care for CGD. Prophylactic trimethoprim-sulfamethoxazole (TMS-SMZ), IFN-γ, or itraconazole proved to significantly reduce the number and severity of infections per patient per year. However, despite dramatic reduction, life-threatening infections still occur; pneumonias, abscesses, adenitis, and osteomyelitis (OM) are frequently reported complications. Bone infections affect ∼1 of every 4 patients with CGD; Serratia and Aspergillus species are the most frequent isolates [2–5]. As mentioned above, patients with CGD frequently show altered inflammatory responses. Patients often do not display symptoms commensurate with the extent of their disease and may present for care late in the course of infection. In addition, noninfectious granulomata, a clinical manifestation of immune dysregulation, are also described in patients with CGD [6–10]. Interestingly, defined macroscopic and microscopic patterns have been described to suggest CGD diagnosis in some of these conditions [6–8].

Patients, materials, and methods. Since 1987, 46 patients received a diagnosis of CGD and were observed at the Immunology Unit of the Hospital Nacional de Pediatría J. P. Garrahan (Buenos Aires, Argentina). CGD diagnosis was based on nitroblue tetrazolium reduction and/or dihydrorhodamine oxidation tests.

Patients with CGD and OM were selected for this study (case patients). OM was defined as the presence of nontraumatic bone lesions as detected by radiography, CT, or scintigraphy in an infectious diseases clinical setting. If bone samples were available, microbiology and histopathology studies were performed. Histopathology slides were reviewed by a single experienced pathologist (M.L.G.) for the presence or absence of necrotic bone, remodeled bone, granulomata, fibrosis, granulation tissue, tissue necrosis, and abscesses; types of cellular infiltration (neutrophils, eosinophils, lymphocytes, histiocytes, osteoclasts, plasma cells, and multinucleated giant cells); and inflammatory pattern(s) (acute, chronic, or acute and chronic) [11]. The presence of necrotic bone and any type of inflammation were considered necessary for histopathologic diagnosis of OM. Clinical charts of these patients were evaluated for clinical OM as part of the first infection attributable to CGD and prompting its diagnosis, fever at the time of OM diagnosis, single or multifocal bone involvement, multiorgan involvement, outcome, and prophylaxis at the time of OM) and laboratory (WBC, neutrophil, and platelet counts; hematocrit; hemoglobin concentration; and erythrocyte sedimentation rate [ESR]) manifestations at the time of OM diagnosis and after treatment was completed. Microbiology results of bone samples were also analyzed.

The control group was defined by a histopathologic diagnosis of OM in patients without CGD. All information was reviewed in the same way as for the case patients, and results for the 2 groups were compared. Individuals with other primary or secondary immunodeficiency were excluded from the analysis.

For nominal variables, χ² or Fisher’s exact test (depending...
On the sample size) was applied; for continuous variables, Student’s t test was used. For those variables with statistically significant differences between case and control groups, the OR, 95% CI, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were also calculated. This study was approved by the Research Committee and by the Ethics in Investigation Board of the Hospital Nacional de Pediatría J. P. Garrahan (research protocol, 452/07).

Results. Among our case patients, 14 of 46 patients with CGD in our cohort received a diagnosis of OM (11 were X linked, 1 was autosomal recessive, and 2 had an undetermined inheritance pattern). Of the 14 patients, the median age at OM diagnosis was 4 years and 11 months (range, 1 year and 5 months to 11 years and 11 months), 7 patients presented with multifocal involvement, and 11 showed other organ involvement. Lower extremities were the most frequently affected sites (8 OM foci), followed by the chest wall (6 foci), upper extremities (5 foci), and the head (2 foci). With regard to other site involvement, lungs were affected in 5 cases, skin in 4, CNS and lymph nodes in 2, and joints in 1 (1 patient had simultaneous lung and CNS involvement, another had skin and joint involvement, and a third had skin and lymph node involve-
significant differences in total WBC, neutrophil, and platelet counts or even in ESR were detected between the 2 groups at OM diagnosis or after treatment completion. Hematocrit was significantly lower in patients with CGD at diagnosis (median for case patients, 28.7%; median for control subjects, 32.9%; \( P = .03 \)) but not after OM was cured. No statistical differences between groups were evident for fever or age at OM diagnosis.

Histopathologic findings for case patients and control subjects were also compared. The presence of acute and chronic inflammation on the slides of OM samples was significantly underrepresented among case patients (\( P = .05 \); OR, 0.13; 95% CI, 0.02–0.81; sensitivity, 0.17; specificity, 0.65; PPV, 0.13; NPV, 0.41). This was the only single variable comparison that was statistically different between groups. When the inflammatory patterns were combined with the other variables, statistically significant differences arose (table 1 and figure 1). The presence of chronic inflammation plus granulomata, multinucleated giant cells, histiocytes, or necrosis was significantly overrepresented among case patients. On the other hand, the presence of acute and chronic inflammation plus granulation tissue, remodeled bone, or lymphocytes was significantly underrepresented and poorly associated with CGD-affected patients with OM (table 1).

**Discussion.** Our findings of incidence and pathogen distribution in CGD-affected patients with OM are in accordance with other published reports [2, 13–16]. Fourteen (30%) of our 46 patients with CGD presented with OM during their follow-up. Indeed, OM by itself or as part of a more widely distributed infection was the first manifestation attributable to CGD and prompted its diagnosis in almost 17% (8 of 46) of our patients.

*S. marcescens* (in 7 patients [54%]) and *Aspergillus* species (in 5 patients [38%]) were the most prevalent microorganisms found, accounting for >90% of the microbiologically positive samples tested. Interestingly, none of these microorganisms were found in the control group or even in any other bone sample processed at our center (for cases vs. controls *S. marcescens* OM, \( P < .001 \); for *Aspergillus* species OM, \( P = .007 \)). This indicates that *S. marcescens* and *Aspergillus* species, when isolated from bone lesions, are by themselves highly suggestive of CGD diagnosis. *Penicillium piceum*, the other isolate among case patients, is a rare filamentous fungus-producing pathology in immunocompromised patients [12].

Treatment for *S. marcescens* OM was based on antibiotics plus surgical debridement, resulting in a successful outcome in all patients. On the other hand, *Aspergillus* species OM with lung involvement was less aggressively treated and resulted in death in 5 of 5 patients. Aggressive and timely therapeutic policies should be pursued in CGD-affected patients with fungal OM, to prevent poor outcomes [17].

At the time of OM onset, anti-infective prophylaxis measures were inconsistent and not under full adherence by our patients.
None of the 6 patients with a previous diagnosis of CGD was receiving IFN-γ therapy; 5 were treated with antibacterial (TMS-SMZ), and 3 were treated with antifungal (itraconazole or voriconazole) prophylaxis. Although no fungal OM presented in patients receiving antifungal prophylaxis, TMS-SMZ–resistant S. marcescens infections still occurred in 3 of 5 patients receiving TMS-SMZ prophylaxis. Because OM is a highly prevalent complication among patients with CGD, and because S. marcescens is its most prevalent causative agent, it would be worth considering new antibacterial prophylactic regimens that cover TMS-SMZ–resistant S. marcescens (e.g., fluoroquinolones) [18].

To determine OM-related suggestive markers for CGD, we defined a comparative control group. Multifocal OM and/or simultaneous other-organ involvement were the most relevant and statistically significant clinical findings suggesting CGD as an underlying disease.

Even though patients with CGD had increased ESR, WBC, neutrophil, and platelet counts at OM onset, differences were not statistically significant when compared with those of control subjects. On the other hand, case patients had significantly lower hematocrits than did control subjects at OM diagnosis. This difference, which disappeared after treatment completion, is probably a reflection of recurrent, long-term, or more-severe infections in immunodeficient than in immunocompetent patients [19].

On the comparative analysis of OM histopathology slides, we searched for the presence of different types of bone lesions, cellular infiltrates, and inflammatory patterns. Interestingly, there was a critical difference in inflammatory patterns between case patients and control subjects. To rule out the possibility that the CGD-associated histopathology patterns were related to the germs rather than to CGD, special emphasis was placed on the search of patients without CGD who had OM caused by S. marcescens or Aspergillus species. No such cases were diagnosed in our center, and the medical literature is limited on this topic. Thus, even if we cannot completely exclude the possibility that the particular histopathologic patterns described in CGD-OM–affected patients are germ dependent rather than CGD dependent, the simultaneous finding of these germs and histopathology findings is indeed significantly associated with CGD with a very high specificity and PPV. As mentioned above, CGD is not only characterized by increased susceptibility to infectious diseases, but its altered inflammatory mechanisms are also a constitutive part of this entity. Thus, particular inflammatory patterns associated or not associated with certain types of infections should be considered a rule rather than an exception in patients with CGD [6–10, 20–22].

In summary, we clearly define here that OM—when multifocal, associated with other-organ involvement, produced by S. marcescens or Aspergillus species, or with histopathology signs of chronic inflammation and granulomata-related features—is significantly associated with CGD and should raise the suspicion of the presence of CGD as an underlying condition. In these patients, anti-infective prophylactic measures aiming to cover highly prevalent microorganisms, as well as aggressive therapeutic measures, should be strongly encouraged [3–5, 17, 18].

Acknowledgments

We thank Dr. Patricia Santos and Dr. Lidia Casimir for their collaboration in the processing of microbiology specimens.

Financial support. National Institutes of Health and Fogarty International Center and the Fogarty International Research Collaboration Award (R01TW006644 to S.D.R.).

Potential conflicts of interest. All authors: no conflicts.

References


