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


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Herein, we describe a combination of clinical, microbiologic, and histopathologic findings significantly associated with osteomyelitis in chronic granulomatous disease. When present, these features should raise the suspicion of underlying chronic granulomatous disease. In patients with these findings, anti-infective prophylactic measures aiming to cover highly prevalent microorganisms, as well as aggressive therapeutic measures, should be strongly encouraged.

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
Table 1. Histopathologic data comparison and statistical analysis for case patients and control subjects.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Acute inflammation</th>
<th>Chronic inflammation</th>
<th>Acute and chronic inflammation</th>
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<tbody>
<tr>
<td>Granulomata</td>
<td>NS</td>
<td>.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NS</td>
</tr>
<tr>
<td>Histiocytes</td>
<td>NS</td>
<td>.007&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NS</td>
</tr>
<tr>
<td>MnGC</td>
<td>NS</td>
<td>.007&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NS</td>
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<tr>
<td>Osteoclasts</td>
<td>NS</td>
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<tr>
<td>Lymphocytes</td>
<td>NS</td>
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<tr>
<td>Plasma cells</td>
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<td>NS</td>
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<td>Neutrophils</td>
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<td>Eosinophils</td>
<td>NS</td>
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<tr>
<td>Tissue necrosis</td>
<td>NS</td>
<td>.001&lt;sup&gt;d&lt;/sup&gt;</td>
<td>NS</td>
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<tr>
<td>Fibrosis</td>
<td>NS</td>
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<td>Abscesses</td>
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<td>NS</td>
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<tr>
<td>Granulation tissue</td>
<td>NS</td>
<td>NS</td>
<td>.05&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Remodeled bone</td>
<td>NS</td>
<td>NS</td>
<td>.04&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

NOTE. CGD, chronic granulomatous disease; MnGC, multinucleated giant cells; NPV, negative predictive value; NS, not statistically significant; PPV, positive predictive value.

<sup>a</sup> OR and 95% CI, <i>p</i>; sensitivity, 0.3; specificity, 1; PPV, 1; NPV, 0.74.

<sup>b</sup> OR and 95% CI, <i>p</i>; sensitivity, 0.4; specificity, 1; PPV, 1; NPV, 0.77.

<sup>c</sup> OR and 95% CI, <i>p</i>; sensitivity, 0.4; specificity, 1; PPV, 1; NPV, 0.77.

<sup>d</sup> OR and 95% CI, <i>p</i>; sensitivity, 0.5; specificity, 1; PPV, 1; NPV, 0.8.

<sup>e</sup> OR, 0.13; 95% CI, 0.22–0.81; sensitivity, 0.2; specificity, 0.35; PPV, 0.13; NPV, 0.47.

<sup>f</sup> OR, 0.16; 95% CI, 0.02–0.99; sensitivity, 0.2; specificity, 0.4; PPV, 0.14; NPV, 0.5.

*NOTE.* CGD, chronic granulomatous disease; MnGC, multinucleated giant cells; NPV, negative predictive value; NS, not statistically significant; PPV, positive predictive value.

on the sample size) was applied; for continuous variables, Student’s <i>t</i> 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 involvement). At least 1 bone lesion per patient was surgically sampled, either for diagnostic or therapeutic reasons. All samples were microbiologically analyzed, and 12 of 14 were histopathology evaluated. Ten of 12 of these specimens were confirmed to have OM (no necrotic bone could be found on the other 2 samples). <i>Serratia marcescens</i> was isolated from 7 specimens, different species of <i>Aspergillus</i> grew from 5 (<i>Aspergillus fumigatus</i> in 3, <i>Aspergillus flavus</i> in 1, and another <i>Aspergillus</i> species in 1), <i>Penicillium piceum</i> was isolated from 1, and 1 specimen had negative microbiology results. All patients with fungal infections (6 of 6) had multiorgan involvement and a poorer outcome; all deaths (5) occurred in fungi-infected patients. Treatment for fungal OM was based mainly on antifungal drugs (6 of 6) and surgery (2 of 6). The only survivor of a fungal OM was a patient who presented with a lung nodule and a rib lesion produced by <i>P. piceum</i>. He was aggressively and successfully treated with surgery and antifungals [12]. In contrast, 5 of 7 bacteria-infected patients had other-organ involvement, but no deaths were seen in this group. All 7 patients with <i>S. marcescens</i> infections were treated with antibiotics plus surgery, and they were cured. OM was part of the first infection attributable to CGD and prompted its diagnosis in 8 of 14 patients (4 with <i>S. marcescens</i> and 4 with <i>Aspergillus</i> species). None of these patients were receiving anti-infective prophylaxis; neither was 1 patient who had received a previous diagnosis of CGD. None of the patients receiving antifungal prophylaxis developed fungal OM. Of the 7 patients presenting bacterial OM, 3 were receiving antibacterial prophylaxis, whereas the remaining 4 were not.

Twenty consecutive patients with histopathologically confirmed OM and without CGD were analyzed as our control group. Neither primary or secondary immunodeficiency nor data suggestive of CGD emerged from the analysis of their clinical charts by an experienced immunologist (S.D.R.). Microbiology analysis was performed on all bone samples. Six specimens had positive test results for <i>Staphylococcus aureus</i> (4 methicillin resistant and 2 methicillin sensitive), and 4 samples grew mycobacteria (2 <i>Mycobacterium tuberculosis</i> and 2 <i>Mycobacterium bovis BCG</i> strain). Enterobacter species was cultured from 2 specimens; <i>Staphylococcus epidermidis</i>, <i>Streptococcus viridans</i>, <i>Pseudomonas aeruginosa</i>, and <i>Escherichia coli</i> were isolated from 1 sample each, and 4 of the specimens had negative test results. Clinical and laboratory features were compared between case patients and control subjects. Multifocal OM was present in 7 of 14 patients with CGD, compared with 1 of 20 control subjects (<i>P</i> = .004; OR, 19; 95% CI, 1.96–183.44; sensitivity, 0.5; specificity, 0.95; PPV, 0.87; NPV, 0.73), and other-organ involvement was detected on 11 of 14 case patients and in only 1 of 20 control subjects (<i>P</i> < .001; OR, 69; 95% CI, 6.43–754.19; sensitivity, 0.78; specificity, 0.95; PPV, 0.92; NPV, 0.86). No
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 granulomas, 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.

Figure 1. Osteomyelitis histopathology patterns in patients with chronic granulomatous disease. A wide spectrum of granuloma-associated histology features were seen in our patients. A, Low-power field picture in which multiple and full-blown granulomas (G) with central abscedation and necrosis are seen. A multinucleated giant cell is also detected (black arrow). Upper left, Necrotic bone lesion (NB) surrounded by tissue necrosis of the same patient (hematoxylin-eosin stain; original magnification, ×250). B, Subtle granuloma structure, mainly conformed by epitheliod histiocytes (black arrows) and lymphocytes (white arrows) (hematoxylin-eosin stain; original magnification, ×400). C, Necrotic bone lesion (NB) in the setting of mild fibrosis (F) and chronic inflammation (CI) (hematoxylin-eosin stain; original magnification, ×400).
None of the 6 patients with a previous diagnosis of CGD was receiving IFN-\(\gamma\) 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].

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References


