Prevalence of Howell-Jolly Body-Like Inclusions in HIV Patients and Their Correlation with CD4 Counts and HIV RNA Viral Load

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Abstract. Previous reports have described the rare occurrence of detached nuclear fragments resembling Howell-Jolly bodies within neutrophils from HIV patients, organ-transplant recipients, and patients on immunosuppressive drugs. To date, their potential clinical significance is unknown, and pathologists tend to disregard their presence. Our study sought to find a correlation between these inclusions and the overall disease state, specifically within the HIV patient population. Eighty-three peripheral smears, all from different patients, were examined for the presence of inclusions and compared with recent CD4 counts and HIV RNA viral loads. Six cases contained inclusions, yielding a prevalence of 7.2%. These six patients had a mean CD4 count of 546±305 cells/μL compared to 247±242 cells/μL in those lacking inclusions (p<0.006) and viral loads of 1,686±3,446 copies/mL compared to 241,882±1,137,229 copies/mL in those lacking inclusions (p=0.6). These findings indicate that the presence of Howell-Jolly body-like inclusions may be viewed as a potential biomarker indicative of a low risk for disease progression and/or good response to therapy based upon higher CD4 counts and relatively favorable viral loads.

Introduction

Howell-Jolly bodies are a well-known entity found in red blood cells. They are nuclear fragments, composed of deoxyribonucleic acid, commonly observed in the peripheral blood smears of hyposplenic or asplenic patients [1]. Recently, similar inclusions often referred to as Howell-Jolly body-like inclusions (HJBLIs) have been reported in the neutrophils of patients with acquired immune deficiency syndrome (AIDS) [2,3,4,5]. Beyond their association with immunosuppression, the clinical significance of these inclusions has not been well described. In this study, we sought to determine whether the presence of HJBLIs, specifically within a patient population with human immunodeficiency virus (HIV) or AIDS, could be considered a prognostic indicator of disease severity and/or response to treatment.

Materials and Methods

Over a seven-month period beginning in late 2011, patients with a positive HIV antibody test or documented AIDS in their medical record, and who had accompanying flow cytometry data for CD4 counts and a peripheral smear made for pathology review were selected for inclusion in the study. The smears were prepared using the Wright-Giemsa stain, and all were dated within thirty days of the corresponding CD4 count. Associated viral loads were also available.

Each slide was then examined under oil immersion for the presence of round, densely basophilic inclusions at least 1 μm in diameter within the cytoplasm of segmentated or band neutrophils that were not in continuity with the nucleus (Figure 1). A minimum of 100 neutrophils per slide were examined by both a faculty pathologist and a resident, and mutual agreement was required to deem a smear as having a Howell-Jolly body-like inclusion.

The CD4 count and the HIV RNA viral load were chosen as serologic indices to risk-stratify each patient’s current disease state. CD4 counts were determined by flow cytometry using the BD FACSCalibur, and HIV RNA viral loads were measured by the Roche COBAS AmpliPrep instrument and the Roche COBAS TaqMan HIV-1 Real-time PCR System.
The unpaired t-test was used to assess differences in CD4 counts and HIV RNA viral loads between the group with HJBLIs and those without.

**Results**

A total of 83 peripheral blood smears (from unique individuals) with accompanying laboratory values were included in the analysis. Six smears contained HJBLIs, yielding a prevalence of 7.2% in our study. In the group with these inclusions (Table 1), all but one had CD4 counts above 200 cells/μL and HIV viral loads of less than 850 copies/mL. Only one patient was found to have a low CD4 count paired with a relatively elevated viral load (60 cells/μL and 8690 copies/mL, respectively). Among these six patients, the overall means and standard deviations for CD4 count and HIV RNA viral load were 546±305 cells/μL and 1,686±3,446 copies/mL, respectively.

In contrast, 77 patients did not have any definitive evidence of HJBLIs within their neutrophils (Table 2). These patients represented a wide and highly variable spectrum of serologic profiles. CD4 counts ranged from 3 to 991 cells/μL with a mean and standard deviation of 247±242 cells/μL (p<0.006 when compared to those with HJBLIs). The HIV RNA viral loads ranged from “undetectable” to 9,610,000 copies/mL with a mean and standard deviation of 241,882±1,137,229 copies/mL (p=0.6 when compared to those with HJBLIs).

**Discussion**

As early as 1989, Bain postulated that HJBLIs were associated with dysplastic granulopoiesis in immunocompromised patients and those undergoing chemotherapy [6].

Slagel et al later observed what they described as “discrete, densely basophilic inclusions” in the neutrophils of 3 of 25 (12%) AIDS patients in their 1994 study [2]. The group further characterized the inclusions via differential staining. The Gram, periodic-acid-Schiff, and Gomori methanamine silver stains failed to highlight the bodies but they did demonstrate positivity with the Feulgen reaction, suggesting that they were indeed fragments of DNA like their namesake in the red blood cell. The group also utilized electron microscopy and found them to be indistinguishable from the neutrophil’s nuclear lobes. Their hypothesis was further corroborated by Ong et al, who arrived at the same conclusion – namely, that these dense basophilic bodies represented DNA - by employing 4′, 6-diamidino-2-phenylindole (DAPI), a reagent that strongly binds DNA and emits a blue fluorescence [7].

In addition to Dr. Slagel’s group, Goodwin et al reported on their experience of encountering HJBLIs in a small series of AIDS patients with Mycobacterium avium complex infection [4]. A few years later, they further elaborated on their observations by stating that at their institution (with only rare exceptions) the inclusions were found exclusively in patients with known HIV seropositivity and were even discovered in neutrophil precursors such as myelocytes [5].

Beyond the association with HIV and AIDS, Abdel-Monem et al reported a case of HJBLIs in a post-heart transplant patient who contracted a cytomegalovirus infection and was subsequently treated with gancyclovir [8]. The patient had been on appropriate anti-rejection medications but the inclusions only appeared soon after initiation of gancyclovir and were only present for a few days. Given the temporal relationship between the appearance of the inclusions and gancyclovir, the authors postulated that the inclusions were directly associated with taking the medication.
Another case involving organ transplant was reported by Kahwash et al in a patient who had previously undergone lung transplant and was therefore also receiving anti-rejection medications [9]. The possible link between HJBLIs and immunosuppressive medications was also highlighted by a patient with mantle cell lymphoma who had received several cycles of chemotherapy with a number of alkylating agents, steroids, and rituximab [7]. However, to date, no data has been presented in the literature to suggest a possible prognostic relationship between HJBLIs on a patient’s peripheral blood smear and their disease. Our focus was solely on immunocompromised (secondary to HIV infection and AIDS) patients. While Slagel et al did present valuable information, they were not able to provide comprehensive or easily monitored data-points from which prognostic conclusions could be drawn. In terms of clinical outcomes, they provided each patient’s history of opportunistic infections in addition to whether the patient was living or dead at the time the study was submitted, but from this information alone, it is difficult to draw any clear associations between the HJBLIs and the disease state at the time the inclusions were found. For example, we do not know whether the inclusions were temporally related to any particular opportunistic infection or whether the nature of death was a direct complication of severe HIV infection.

In our attempt to find a surrogate measure of mortality and morbidity that could be easily correlated with the detection of HJBLIs on peripheral blood smears, we opted to analyze two widely accepted and regularly followed laboratory values with known prognostic significance: the CD4 count and the HIV RNA viral load. In fact, current treatment guidelines put forth by the United States Department of Health and Human Services state that: “HIV-infected patients with CD4 counts <200 cells/μL are at higher risk of opportunistic diseases, non-AIDS morbidity, and death than HIV-infected patients with higher CD4 counts...[and] measures of viral replication have been known to predict HIV disease progression. Among untreated HIV-infected individuals, time to clinical progression and mortality is fastest in those with greater viral loads.”[10]

Under these assumptions regarding the utility of monitoring CD4 count and HIV RNA viral load, our data suggests that, when present, HJBLIs are more likely to be found in the peripheral blood smears of patients with relatively higher CD4

<table>
<thead>
<tr>
<th>Patient</th>
<th>CD4 (cells/μL)</th>
<th>HIV RNA copies (copies/mL)</th>
<th>Viral load Log10</th>
<th>HAART Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>710</td>
<td>94</td>
<td>2</td>
<td>atazanavir, ritonavir, emtricitabine</td>
</tr>
<tr>
<td>Patient 2</td>
<td>556</td>
<td>&lt;48</td>
<td>&lt;1.68</td>
<td>nevirapine, lamivudine, didanosine</td>
</tr>
<tr>
<td>Patient 3</td>
<td>966</td>
<td>Undetectable</td>
<td>&lt;1.3</td>
<td>tenoforv, emtricitabine, etravirine</td>
</tr>
<tr>
<td>Patient 4</td>
<td>390</td>
<td>849</td>
<td>2.9</td>
<td>None</td>
</tr>
<tr>
<td>Patient 5</td>
<td>60</td>
<td>8690</td>
<td>4</td>
<td>None</td>
</tr>
<tr>
<td>Patient 6</td>
<td>593</td>
<td>435</td>
<td>2.6</td>
<td>Data not available</td>
</tr>
</tbody>
</table>

Table 2. Patients without HJBLIs (Howell-Jolly Body Like Inclusions).

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Mean ± SD</th>
<th>Compared to Patients with HJBLIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 count (cells/μL)</td>
<td>3–991</td>
<td>247±243</td>
<td>P&lt;0.006</td>
</tr>
<tr>
<td>HIV RNA (copies/mL)</td>
<td>Undetectable – 9,610,000</td>
<td>241,882 ± 1,137,229</td>
<td>P&gt;0.6</td>
</tr>
<tr>
<td>Viral load Log10</td>
<td>&lt;1.3–7.0</td>
<td>3.6±1.6</td>
<td></td>
</tr>
</tbody>
</table>
counts and lower HIV RNA viral loads. Five out of six cases with inclusions had a CD4 count well above 200 cells/μL. However, the more striking finding was that the mean CD4 count in all six was more than twice as high, and statistically significant when compared to those in which HJBLIs were not found. In terms of HIV RNA viral load, the mean number of copies was over 143 times greater in those without HJBLIs, but there was also much more variation in these values, such that no statistical significance could be proven. In absolute terms, however, in over 45% of cases lacking HJBLIs the viral loads were >10,000 copies/ml and a full 25% of cases had levels > 100,000 copies/ml. Therefore, it can be argued that there appears to be a trend toward increased viral loads when HJBLIs are not present.

Besides the sheer magnitude of the viral load standard deviation, the overall low prevalence of HJBLIs further limited our ability to make definitive conclusions about any relationship between the presence of the inclusions on peripheral smear and the patient’s HIV serology results. The use of only CD4 counts and HIV RNA viral load as clinical endpoints may also be limiting. Although widely studied and used clinically, they alone do not definitively represent or predict how well any particular individual’s disease may be progressing or responding. Another confounding factor may be related to medication effect. Among the group with HJBLIs, three patients were on highly active anti-retroviral therapy (HAART) with combination reverse transcriptase inhibitors and protease inhibitors. Two patients were not on any HAART, including the sole patient with unfavorable laboratory values, while data was not available on the last patient. The exact association between anti-viral drugs and HJBLIs has not yet been well delineated (and was not the goal of our study here), but their potential effect on granulopoiesis cannot be discounted. Although the exact mechanism that produces these HJBLIs remains unknown, the results of our study on these inclusions seem to suggest that they coincide with increased numbers of helper T-cells that are necessary to maintain the body’s immune response, and to a lesser extent demonstrate reduced viral replicative activity. In this light, HJBLIs may be viewed as a morphologic feature evident in the peripheral blood, signifying a good prognosis and/or a decreased risk for disease progression. The strength of this correlation with HIV infection and disease progression still leaves room for debate; however, with further study, the prospect of finding a correlation and its potential implication remain very intriguing.

References