

# A Critical Reappraisal of Prolonged Neutropenia as a Risk Factor for Invasive Pulmonary Aspergillosis

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Prolonged neutropenia is generally thought to be the major factor for invasive pulmonary aspergillosis (IPA). In the present study, we characterize the frequency, severity, and duration of neutropenia that immediately precedes IPA. Prolonged neutropenia was identified in only one third of all IPA cases and occurred exclusively in hematologic patients.

**Keywords.** *Aspergillus*; immunosuppression; invasive aspergillosis; invasive fungal infection; neutropenia.

Twenty years ago, Gerson et al [1] demonstrated the importance of prolonged neutropenia (PN) as a risk factor for invasive pulmonary aspergillosis (IPA). The notion that PN is the major factor for IPA is a view that persists in textbooks and review articles [2]. Although PN strongly predisposes to IPA, our clinical experience called into question the current relative importance of PN. A reappraisal of the importance of PN is clinically important for at least 2 reasons. First, the identification of novel risk factors for IPA has, by definition, diluted the relative importance of PN. Second, the widespread use of previously unavailable diagnostic modalities has broadened the clinical spectrum of IPA by allowing for the identification of patients with IPA who would have previously gone unrecognized. Our goal in the present study was to characterize the frequency, severity, and duration of neutropenia that immediately precedes the diagnosis of IPA. In addition, in view of the fact that corticosteroids (CS) are known to have a major impact on both neutrophil number and function [3], we also analyzed CS use in these patients.

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## METHODS

Patients hospitalized at Massachusetts General Hospital or Brigham and Women's Hospital from 2004 to 2014 were retrospectively identified with approval from the Partners Healthcare Institutional Review Board. Patients were included if European Organization for Research and Treatment of Cancer (EORTC) criteria for probable or proven IPA [4] were met and a peripheral blood absolute neutrophil count (ANC) was drawn within 3 months before IPA diagnosis. Neutropenia was defined as ANC < 500. The duration of neutropenia was calculated for all patients with ANC < 500 at least once during the 3 months before IPA onset. The onset of neutropenia was defined as the first day in which ANC was < 500. The final day of a neutropenic episode was marked by either the onset of IPA or 2 consecutive ANC values > 500. Due to the retrospective nature of our study, we did not have access to a complete set of ANC values before IPA onset. As a result, we were unable to determine the precise date of onset of neutropenia for some patients. When the date of onset of neutropenia could not be determined precisely, the duration was recorded as the range of possible days. The final day of neutropenia was marked by either the onset of IPA or 2 consecutive ANC > 500. Neutropenia was categorized as prolonged when > 10 days. Galactomannan was considered positive when  $\geq 0.5$  in serum or bronchoalveolar lavage specimen. Profound neutropenia was defined as ANC < 50. Patients were classified into the following categories of immunosuppression: hematologic malignancy or bone marrow transplant (HM/BMT), solid organ transplant (SOT), or Other. One patient met criteria for both HM/BMT and SOT. Nine years after kidney transplantation, the patient was diagnosed with a hematologic malignancy. He was initiated on chemotherapy and developed IPA 2 months later. Due to the temporal association between initiation of chemotherapy and IPA, the patient was analyzed in the HM/BMT group. For each patient, the medical record was reviewed for details regarding CS administration during the 6-month period before IPA onset. Corticosteroid exposure, expressed in milligrams of prednisone equivalents, was calculated as the average daily dose during the 3-weeks before IPA onset. Prolonged CS use was defined as CS use  $\geq 0.3$  mg/kg per day for  $\geq 3$  weeks. The data are presented as medians with interquartile ranges and as frequencies with percentages for continuous and categorical variables, respectively. Continuous variables were compared with the Mann-Whitney *U* test; categorical variables were compared with either Fisher's exact test ( $n < 5$ ) or  $\chi^2$  ( $n \geq 5$ ) test.

## RESULTS

In total, 324 patients were included in the present study (201 HM/BMT, 41 SOT, and 82 Other). Demographic details of

**Table 1. Characteristics of Patients With Invasive Pulmonary Aspergillosis<sup>a</sup>**

Patient Characteristics	Total (n = 324)	HM/BMT (n = 201)	SOT (n = 41)	Other (n = 82)	P Value HM/BMT vs SOT	P Value HM/BMT vs Other	P Value SOT vs Other
<b>Demographics</b>							
Age, years	59.1 (49.0–66.5)	59.1 (48.0–66.3)	61.1 (46.2–64.4)	58.8 (51.0–69.1)	.69	.33	.38
Diabetes mellitus	24.7% (80/324)	19.9% (40/201)	46.3% (19/41)	25.6% (21/82)	.0003	.29	.021
COPD or Asthma	22.2% (72/324)	13.4% (27/201)	22.0% (9/41)	43.9% (36/82)	.16	<.0001	.017
HIV	3.1% (10/324)	1.0% (2/201)	0.0% (0/41)	9.8% (8/82)	>.99	.002	.069
Splenectomy	2.5% (8/324)	3.0% (6/201)	2.4% (1/41)	1.2% (1/82)	>.99	.70	>.99
Prolonged CS use and/or Prolonged neutropenia	71.9% (220/306)	83.5% (162/194)	47.4% (18/38)	54.1% (40/74)	<.0001	<.0001	.50
<b>Galactomannan</b>							
Serum GM	0.764 (0.150–2.417)	0.913 (0.240–2.382)	0.320 (0.053–0.890)	0.672 (0.045–3.505)	.006	.23	.23
Serum GM ≥ 0.5	59.3% (178/300)	66.1% (125/189)	35.1% (13/37)	54.1% (40/74)	.0004	.068	.06
BAL ≥ 0.5	84.0% (89/106)	78.8% (52/66)	93.8% (15/16)	91.7% (22/24)	.30	.27	.99
<b>Diagnostic Confidence (EORTC Criteria)</b>							
Proven IPA	31.2% (101/324)	26.4% (53/201)	36.6% (15/41)	40.2% (33/82)	.18	.021	.70
Probable IPA	68.8% (223/324)	73.6% (148/201)	63.4% (26/41)	59.8% (49/82)			

Abbreviations: BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; CS, corticosteroid; EORTC, European Organization for Research and Treatment of Cancer; GM, galactomannan; HIV, human immunodeficiency virus; HM/BMT, hematologic malignancy or bone marrow transplant; IPA, invasive pulmonary aspergillosis; IQR, interquartile range; SOT, solid organ transplant.

<sup>a</sup> Data are presented as median (IQR) or n (%).

the patients are shown in Table 1. Based on EORTC criteria, IPA cases were classified as proven in 101 patients (31.2%) and “probable” in 223 patients (68.8%).

Neutropenia (of any duration) preceded IPA in 147 (45.4%) patients and was significantly more common in the HM/BMT (67.7%) cohort compared with SOT (12.2%,  $P < .0001$ ) or Other (7.3%,  $P < .0001$ ). In 5 patients with neutropenia, we were unable to determine whether the neutropenic episode lasted >10 days. Thus, amongst 319 evaluable patients, PN was present in 108 (33.9%). Amongst evaluable patients with proven IPA, PN was identified in 29.6%. Prolonged neutropenia was significantly more common in HM/BMT (54.8%) compared with SOT (0.0%,  $P < .0001$ ) or Other (0.0%,  $P < .0001$ ). No cases of PN were identified in the SOT or Other cohorts (Table 2).

Sufficient data to calculate whether CS use was  $\geq 0.3$  mg/kg per day for  $\geq 3$  weeks were available in 308 patients, of whom 146 (47.4%) had prolonged CS use (Table 3). The prevalence of prolonged CS use was significantly lower in patients with PN compared with those with neutropenia of shorter durations

(29.0% vs 62.5%,  $P < .001$ ). Prolonged neutropenia, prolonged CS, or both were present in 220 (71.9%) of 306 evaluable patients and occurred more frequently in HM/BMT (83.5%) than SOT (47.4%,  $P < .0001$ ) or Other (54.1%,  $P < .0001$ ) (Table 1).

## DISCUSSION

Although early studies on IPA emphasized PN as the most important risk factor for IPA [1], recent data suggest that neutropenia is present in only half of all IPA patients [5]. However, prior studies have differed in the ANC value used to define neutropenia and in the time interval considered before diagnosis. The latter point is important because some studies have defined neutropenia according to the ANC on the day of diagnosis with IPA [5, 6]. This approach underestimates the importance of neutropenia because it fails to include patients with a recent episode of neutropenia that has resolved by the time of IPA diagnosis.

In the present study, we had access to detailed clinical information, including ANC values several months before IPA,

**Table 2. Duration and Severity of Neutropenia That Precedes Invasive Pulmonary Aspergillosis<sup>a</sup>**

Duration and Severity of Neutropenia	Total	HM/BMT	SOT	Other
Any neutropenia	45.4% (147/324)	67.7% (136/201)	12.2%* (5/41)	7.3%* (6/82)
Prolonged neutropenia	33.9% (108/319)	54.8% (108/197)	0.0%* (0/40)	0.0%* (0/82)
Profound neutropenia	37.3% (121/324)	58.2% (117/201)	4.9%* (2/41)	2.4%* (2/82)
Prolonged and/or Profound neutropenia	41.1% (132/321)	64.3% (128/199)	5.0%* (2/40)	2.4%* (2/82)

Abbreviations: ANC, absolute neutrophil count; HM/BMT, hematologic malignancy or bone marrow transplant; SOT, solid organ transplant.

<sup>a</sup> The denominator for the data listed above refers to the number of evaluable patients within each immunosuppressive cohort. Prolonged neutropenia: ANC < 500 for >10 days. Profound neutropenia: ANC < 50.

\*  $P < .0001$  for comparison with HM/BMT.

**Table 3. Corticosteroid Use Before Onset of Invasive Pulmonary Aspergillosis<sup>a</sup>**

Corticosteroid Use	Total	HM/BMT	SOT	Other	P Value HM/BMT vs SOT	P Value HM/BMT vs Other	P Value SOT vs Other
Any CS use	67.4% (217/322)	60.0% (120/200)	92.7% (38/41)	72.8% (59/81)	<.0001	.043	.01
Prolonged CS use	47.4% (146/308)	44.1% (86/195)	48.7% (19/39)	55.4% (41/74)	.60	.097	.50

Abbreviations: CS, corticosteroid; HM/BMT, hematologic malignancy or bone marrow transplant; SOT, solid organ transplant.

<sup>a</sup> The denominator for the data listed above refers to the number of evaluable patients within each immunosuppressive cohort. Prolonged CS use:  $\geq 0.3$  mg/kg per day for  $\geq 3$  weeks.

which allowed for a more granular understanding of the frequency, severity, and duration of neutropenia that predisposes to IPA. Neutropenia preceded the onset of IPA in fewer than half of all cases. Prolonged neutropenia was identified in one third of all patients (Table 2). Non-HM/BMT groups comprised 38.0% of all IPA cases, but these groups contributed only 7.5% of neutropenic with no cases of PN. Even amongst hematologic patients, the prevalence of neutropenia was less than anticipated, occurring in just over half of patients. This finding has important clinical implications. First, risk factors other than neutropenia appear to play an important role in predisposing the host to IPA. In our study, amongst hematologic patients, prolonged CS use was significantly more prevalent in patients without neutropenia compared with their neutropenic counterparts. Although a steroid-induced neutrophilic leukocytosis is one plausible explanation [7], the observed relationship between CS use and neutropenia applied only to the hematologic group and, in fact, the inverse relationship was observed in non-hematologic patients. Second, the absence of neutropenia should not dissuade the clinician from considering the diagnosis of IPA. This is particularly applicable to the nonhematologic patient, because PN was not found to precede the diagnosis of IPA in this population.

## CONCLUSIONS

In most studies [5, 6], neutropenia is reported as a risk factor for IPA if ANC < 500 at the time of IPA diagnosis. This approach fails to include patients in whom the ANC has only recently increased above 500 after a period of neutropenia. Our review of all ANC values during a 3-month period before IPA onset allowed us to determine the prevalence of neutropenia with greater resolution than prior reports and represents a major strength of the present study. Furthermore, our ability to confidently rule out neutropenia was strengthened by our decision to exclude patients without a pre-IPA ANC value. For the present study, we used the EORTC case definition of IPA. These criteria classify a case as probable IPA only in the presence of a well defined risk factor for invasive

fungal infection. Because clinicians are taught that patients with neutropenia are predisposed to IPA, diagnostic tests for IPA are likely used more frequently in neutropenic patients compared with those without neutropenia. The fact that nonneutropenic IPA cases might be more likely to go undiagnosed has the potential to overestimate the prevalence of neutropenia in patients with IPA. In our cohort, 10 patients failed to meet inclusion criteria solely on the basis of their absence of EORTC risk factor criteria. We conclude that although prolonged neutropenia is widely recognized as the major risk factor for IPA, ANC < 500 for >10 days was identified in only one third of patients with IPA in our study. We believe that this finding should lead to a reassessment of the risk factors for IPA and open the way to a broader approach to consideration of the diagnosis of this disease in immunosuppressed patients presenting with pneumonia.

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