

Estimation of the effect of neutropenia on rates of clinical bacteraemia in HIV-infected patients

J. CAPERNA, R. E. BARBER, J. G. TOERNER AND W. C. MATHEWS*

Department of Medicine, UCSD Medical Center, 200 W. Arbor Dr., San Diego, CA 92103-8681 USA

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SUMMARY

A retrospective cohort study was conducted to quantitate the relationship between neutropenia and rates of clinical bacteraemia among adults with HIV infection receiving medical care at one institution between 1991–5. The primary exposure, absolute neutrophil count (ANC), was summarized as mean ANC within a given week, using a five-level stratification (reference > 1000/ μ l). ANC stratum-specific rates of bacteraemia were calculated, by organism type. Linear trend tests were performed to assess dose-response relationship between neutropenia and rates of bacteraemia. The cohort included 1645 patients contributing 26785 patients-weeks and 191 episodes of bacteraemia. The unadjusted effect of neutropenia was most evident for bacteraemia due to *E. coli* (RR 3.4), *Klebsiella pneumoniae* (RR 16.7), and *P. aeruginosa* (RR 10.4). For bacteraemia due to any of these three organisms (47 episodes), with reference ANC > 1000/ μ l, relative rates were: 751–1000/ μ l, 1.12; 501–750/ μ l, 2.11; 251–500/ μ l, 13.58; 0–250/ μ l, 21.89.

INTRODUCTION

Neutropenia, induced either by treatment or by endogenous factors, is a well-established risk factor for serious infectious complications [1–3]. In addition, even when an attributable infection cannot be documented, severe neutropenia has been associated with increased risk of febrile episodes requiring empirical antimicrobial therapy and hospitalization [4].

Treatment-induced or endogenous neutropenia, is common among patients with advanced HIV infection. In the absence of cytokine support, 40–50% of patients treated with ganciclovir required treatment interruption due to neutropenia [5] and 16% of late stage zidovudine-treated patients experienced absolute neutrophil counts (ANCs) < 500/ μ l [6]. Among 56 patients with HIV-associated non-Hodgkin's lymphoma, Northfelt [7] reported 99 hospitalizations for febrile neutropenia (ANC < 500/ μ l) within 4 weeks of beginning chemotherapy. In a recent trial of liposomal doxorubicin as single agent therapy of

Author for correspondence.

Kaposi's sarcoma, 42.5% experienced grade IV neutropenia [8].

Patients with advanced HIV infection have a baseline risk of life-threatening infection from a variety of pathogens including capsulated bacteria and *Salmonella* species. The additional risk attributable to the occurrence of neutropenia has been difficult to establish. Some investigators have suggested that neutropenic risk among those with HIV infection may be less than for cancer patients with comparable degrees of neutropenia [2, 9]. Farber and colleagues, [9] compared duration of ANC \geq 1000/ μ l to time with ANC < 1000/ μ l, and reported identical rates of 'non-opportunistic' infections (1.3/1000 person-days) for 30 patients with AIDS and CD4 counts < 200/ μ l. Piliero and colleagues [10], based on retrospective analysis of the experience of a single institution, concluded that, in the absence of an identifiable source, neutropenic patients with HIV infection do not warrant empirical antibiotic therapy.

In contrast, a number of studies have demonstrated an increased risk of bacterial infection in HIV-infected

patients with severe neutropenia [11–14]. Moore and colleagues [15] reported the results of a matched cohort analysis in which the incidence of bacterial infection in 118 HIV-infected patients who experienced neutropenia ($\text{ANC} < 1000/\mu\text{l}$) on one or more occasions was compared to the incidence on 118 non-neutropenic HIV-infected controls matched for CD4 lymphocyte count, use of injecting drugs, and follow-up time. Although no statistically significant associations between neutropenia and individual bacterial complications (bacteraemia, pneumonia, enterocolitis, and infections of normally sterile sites) were demonstrated, for all these infections combined significant associations were demonstrated. Jacobson and colleagues [16] reported the results of a retrospective analysis of hospital utilization for serious bacterial infection as a function of absolute neutrophil count. Based on the results from 2047 patients over a 13-month period, they demonstrated an inverse relationship between the incidence of hospitalization for serious bacterial infection and ANC stratum. Recently, Keiser and colleagues [17] reported the results of a case control study that identified neutropenia ($\text{ANC} < 1000/\mu\text{l}$) as a risk factor for bacteraemia (odds ratio 22.6, $P = 0.028$).

The literature is thus inconsistent. Some assert that neutropenia among patients with HIV infection is both common and associated with somewhat increased risk of infectious complications typical of neutropenia when observed in other populations. Others conclude otherwise. The reviewed studies have differed in eligibility criteria, definition of magnitude and duration of neutropenia, and analytical methodologies. Because we had access to the comprehensive haematology and microbiology histories of an HIV health care cohort at UCSD Medical Center, we sought to determine more precisely the relationship between neutropenia and risk of clinical bacteraemia on a pathogen-specific bases. We conducted a retrospective cohort study to estimate quantitative risk for clinical bacteraemia attributable to time spent in neutropenic periods.

METHODS

Patient selection

The *reference population* was those patients with recognized HIV infection who received medical care at UCSD Medical Center at any time during the study period (14 September 1991–30 June 1995). This population was identified by a database algorithm

based on HIV-specific ICD-9-CM diagnostic codes and patient care locations. From the reference population, an *eligible population* was defined according to the follow criteria: (1) age ≥ 15 years at start of the study period; (2) two or more encounters in the administrative database of the medical centre with at least 30 days follow-up time between first and last encounters. The *study population* was selected from the eligible population conditional on the documentation in the haematology database of at least one complete blood count with differential during the study period.

Study design and data analysis

The study period was partitioned into consecutive 7-day intervals. Members of the study population contributed 7 days of follow-up time to each study week in which at least one ANC could be calculated.

Study weeks during which no ANC determinations were recorded were interval censored. The *outcome of interest* was defined as an *episode of clinical bacteraemia*: culture of a potential bacterial pathogen from blood of a patient whose medical record was over-read by a study physician blinded to ANC level and was judged to represent clinical bacteraemia.

Potential bacteraemia was excluded if it was recurrent within 28 days of a previous clinical bacteraemia due to the same organism. Medical record abstraction was standardized and included ascertainment of the following variables: organism(s) cultured, number of positive cultures; hospital admission; clinical diagnosis of bacteraemia by treating physicians; treatment with antibiotics appropriate for the potential pathogen; presence of an indwelling intravascular catheter on the index date of bacteraemia; survival ≥ 28 days after the index date; and granulocyte colony stimulating factor (GCSF) administration within 28 days prior to the index date or during treatment for the bacteraemia.

Three study physicians reviewed abstraction forms for each potential patient-week of clinical bacteraemia. This involved the independent review of medical record abstraction forms by the study physicians who then categorized the potential bacteraemic weeks as either clinical or non-clinical episodes. The final decision regarding designation of a potential bacteraemia as a clinical event was made by the senior clinician of the study team. Agreement among the physicians reviewing the records was calculated using the kappa statistic, separately com-

paring the pairwise judgements of the other two reviewers to that of the senior clinician. If a medical record could not be located, the potential clinical bacteraemia was considered unconfirmed. The robustness of the results to this assumption was tested by re-analysing the data after designating such 'missing record bacteraemia' as clinical bacteraemia.

The primary independent variable was ANC level during a given study week, categorized into 1 of 5 strata: 0–250, 251–500, 501–750, 751–1000, and > 1000 neutrophils/ μ l. This variable was operationalized as the mean ANC. For study weeks during which no clinical bacteraemia occurred, mean ANC was calculated using all ANC measurements within the 7-day interval. Thus, during each week in which a patient had ≥ 1 ANC determination, the exposure level was assigned to ANC strata corresponding to the mean of all ANC measurements during that study week. For study weeks during which clinical bacteraemia occurred, in order to eliminate temporal ambiguity in the hypothesized causal chain linking neutropenia to risk of bacteraemia, the ANC exposure variables were calculated after censoring eligible ANC within a given study week on the index date of bacteraemia. Thus, for clinical bacteraemic weeks, ANCs measured after the index date, but within the same study week, were excluded from the ANC exposure calculation.

The bacteriology and haematology databases were merged by patient identifier and study week using JMP 3.15 (SAS Institute Inc., Cary, NC, USA). Potential episodes of clinical bacteraemia were assigned to the patient-week in which they occurred and the index date of bacteraemia was taken as the first date within a study week on which a culture was positive for the specified organism or organism category.

The *measure of effect* reported is the rate ratio for clinical bacteraemia comparing lower ANC strata to the reference category of > 1000 neutrophils/ μ l. The patient and study-week matched ANC and bacteriology records were aggregated across the study period to create organism category-specific contingency Table (2×5) representing the joint distributions of neutropenic exposure (five strata) and clinical bacteraemia (present or absent).

Bacteriological studies

Potential pathogens were categorised into nine groups based on preliminary frequency analyses performed

without knowledge of corresponding ANC levels: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, other Gram-negative rods, *Staphylococcus aureus*, other Gram-positive cocci, Gram-positive rods, Gram-negative cocci, and polymicrobial bacteraemia. *E.*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* were included as separate categories because of previous research suggesting that these four organisms accounted for a majority of initial pathogens in previous series [18]. Bacteraemia due to *Staphylococcus epidermidis* was included in the database but was not chart reviewed unless associated with polymicrobial bacteraemia. Linear trend tests to detect a 'dose-response' effect of neutropenia were performed in stratified analyses based on organism categories and pooled using the Mantel–Haenszel procedure implemented in STATA 4.0 (Stata Corporation Inc., College Station, TX, USA) [19]. The conclusions of the pooled analyses were confirmed using exact statistical inference implemented in STATXACT 3 (Cytel Software Corporation, Cambridge, MA, USA).

Although the paper focuses on estimation of unadjusted rates of bacteraemia by ANC strata, some control for confounding due to the presence of indwelling vascular catheters and due to GCSF use was attempted using methods based on the examination of the distribution among the cases of the primary exposure (here neutropenia) by levels of a potential confounder (catheters and GCSF) [20]. By examining the prevalence of neutrophil counts < 500/ μ l across all patient weeks and then separately for weeks associated with the presence or absence of the potential confounder among the cases of bacteraemia, one can infer (without knowing the confounder status of patient weeks without bacteraemia) whether the unadjusted rate ratios could be explained entirely by confounding.

RESULTS

Study population

Between 14 September 1991 and 30 June 1995 (198 weeks), 1645 patients with HIV infection contributed information on ANC. Of the 1645, 173 (10.6%) were female and 196 (12%) were black, 278 (17%) were of Hispanic origin, and 1095 (67%) were white. The median and mean ages were 35 and 36.5 years, respectively (range 15–75 years). The median number of weeks during which each patient had one or more ANC determinations was 10 (interquartile [IQ] range

Table 1. Disposition of potential clinical bacteraemic patient-weeks ($N = 272$), overall and by organism category

	All CB*	Poly	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	Other GNR	<i>S. aureus</i>	Other GPC	GPR
Total	272 (100%)	59 (100%)	17 (100%)	12 (100%)	22 (100%)	46 (100%)	79 (100%)	84 (100%)	36 (100%)
Clinical bacteraemia	191 (70%)	22 (37%)	15 (88%)	12 (100%)	22 (100%)	33 (72%)	62 (78%)	52 (62%)	12 (33%)
Non-clinical bacteraemia	N/A†	34	0	0	0	10	6	30	19
Exclusions	N/A†	2	2	0	0	3	4	2	1
Missing records	N/A†	1	0	0	0	0	7	0	4

* All CB = clinical bacteraemias due to all bacterial organism categories; Poly = polymicrobial clinical bacteraemias (including *S. epidermidis* as eligible); *E. coli* = *Escherichia coli*; *K. pneumoniae* = *Klebsiella pneumoniae*; *P. aeruginosa* = *Pseudomonas aeruginosa*; other species GNR = *Acinetobacter*, *Campylobacter*, *Enterobacter*, *Fusobacterium*, *Klebsiella oxytoca*, *Morganella*, *Prevotella*, *Propionibacterium*, *Proteus*, *Providencia*, *Pseudomonas not aeruginosa*, *Salmonella*, *Serratia*, *Shigella* spp.; *S. aureus* = *Staphylococcus aureus*; Other species GPC = Beta streptococcus, Enterococcus, Micrococcus, Peptostreptococcus, *Streptococcus pneumoniae*, *Streptococcus* other; GPR = *Bacillus*, *Clostridium*, *Corynebacterium*, *Diphtheroids*, *Rhodococcus*.

† Not applicable because of polymicrobial patient-weeks and grouping of multiple organisms within organism categories.

Table 2. Distribution of clinical bacteraemia according to mean ANC recorded in given patient-week

	All CB*	Poly	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	Other GNR	<i>S. aureus</i>	Other GPC	GPR
Total	187 (100%)	22 (100%)	15 (100%)	12 (100%)	22 (100%)	33 (100%)	60 (100%)	51 (100%)	11 (100%)
0–250/ μ l	3	1	0	0	3	0	0	1	0
251–500/ μ l	6	0	2	3	0	0	1	0	0
501–750/ μ l	2	0	1	0	1	0	0	0	0
751–1000/ μ l	4	0	0	0	2	1	0	1	0
> 1000/ μ l	172	21	12	9	16	32	59	49	11

* Refer to note for Table 1 for summary of column abbreviations for organism categories.

4–22), and the mean number of weeks was 16.3 ± 17.6 s.d.

Duration of follow-up

The number of study weeks during which each patient could have contributed ANC exposure information varied and reflected the period of care at the medical centre. The median number of weeks between first and last ANC determinations was 55 (IQ range 18–108) weeks. The percentage of potential weeks during which ANC exposure could have been measured and actually was measured represents the percent completeness of exposure ascertainment for each patient. Thus, for example, if there was a 20-week interval between first and last ANC measurement and if, within that interval there were 10 weeks during which at least one ANC was ascertained, the percent ANC

ascertainment would be 50%. For all patients, the percent ANC ascertainment varied from 1.5–100%, median 27% with interquartile range of 13–56%. There were 26 785 patient weeks during which at least one ANC was recorded in the haematology database.

Bacteriologic outcomes and covariate distributions

During the study period, there were 440 patient-weeks during which positive blood cultures were recorded for at least one organism. Of the 440, 168 were patient-weeks in which only *S. epidermidis* was cultured. Records for these 'S. epi-only' patient-weeks were not reviewed and are not considered further. Of the remaining 272 patient-weeks with positive bacterial blood cultures, after medical record review 191 were judged to include episodes of clinical bacteraemia. These 191 episodes of clinical bacter-

aemia developed in 164 patients, with 17 patients having 2 episodes and 5 having 3 episodes. The median absolute CD4 count among the 140 patients for whom the data were available was 29 (range: 0–643). Table 1 shows the disposition of the 272 potential patient-weeks with clinical bacteraemia, overall and by organism category. Records of all polymicrobial bacteraemia were reviewed. Of the 22 confirmed and eligible polymicrobial clinical bacteraemia, 4 involved *S. epidermidis* as the only other co-pathogen. As noted in the table, the confirmation of clinical bacteraemia after medical record review varied from 33–100% and was highest for *E. coli*, *K. pneumoniae*, and *P. aeruginosa*.

Mortality ascertainment was based on 174/191 (91%). Catheter prevalence was ascertained in 186/191 (97.4%). GCSF use was ascertained in 180/191 (94.2%). Mortality was highest in patients with bacteraemia due to *P. aeruginosa* (40.9%) and lowest among those with *K. pneumoniae* (16.7%). Intravascular catheters were most prevalent among polymicrobial (72.7%) and Gram-positive rod (91.7%) bacteraemia and were present in approximately 50% of patients with bacteraemia due to either *P. aeruginosa* and *K. pneumoniae*. GCSF use varied from 7–33%, by organism category.

Diagnostic reliability

Agreement of the junior physicians who reviewed the records with the judgement of the senior clinician varied by organism category. For potential bacteraemia due to *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, agreement was complete. For *S. aureus*, kappa agreement was complete for one pair of reviewers and 0.88 (s.e. = 0.12) for the other. For the organism category of Gram-positive cocci other than *S. aureus*, pairwise kappa coefficients were 0.67 (s.e. = 0.09) and 0.81 (s.e. = 0.07). For Gram-negative rods other than the three previously mentioned, kappa coefficients were 0.66 (s.e. = 0.15) and 0.49 (s.e. = 0.17). For the combined categories of Gram-positive rods and cocci, agreement coefficients were 0.87 (s.e. = 0.08) and 0.57 (s.e. = 0.08). In most cases of disagreement, the other two reviewers tended to include a bacteraemia as a clinical event where the final judgement was to view it as an unconfirmed bacteraemia. Thus, of 21 disagreements in judgements of one pair of examiners, 14 (67%) were designated clinical bacteraemia when the final determination was unconfirmed. For the other pair of examiners, of 17 disagreements 15 (88%)

Table 3. Score test for trend of rates of clinical bacteraemia with mean ANC stratum (calculated based on ANC measurements within a study week up to and including the index date of bacteraemia),* by organism category

Organism category†	RR‡	Lower	Upper	χ^2	<i>p</i> -value
<i>E. coli</i>	3.354	1.267	8.878	5.940	0.015
<i>K. pneumoniae</i>	16.666	5.059	54.899	21.395	0.000
<i>P. aeruginosa</i>	10.386	4.651	23.192	32.602	0.000
Other GNR	0.673	0.342	1.323	1.319	0.251
GPR	0.835	0.268	2.602	0.096	0.756
<i>S. aureus</i>	0.770	0.470	1.263	1.069	0.301
Other GPC	1.176	0.687	2.014	0.350	0.554

* ANC strata were coded as follows: 1 (> 1000), 2 (751–1000), 3 (501–750), 4 (251–500), and 5 (0–250). Thus, the RR estimate is an approximate estimate of the rate ratio for one unit increase in mean ANC stratum.

† Refer to note for Table 1.

‡ RR estimate, lower and upper 95% confidence limits, and χ^2 test for trend [1 D.F.] Mantel–Haenszel estimate controlling for Organism Category: RR = 1.476, Lower = 1.128, Upper = 1.931, χ^2 = 8.033, *P*-value = 0.005. Approx. χ^2 for unequal RRs (effect modification) 54.74 (6 D.F., *P* = 0.000).

were designated as clinical with the final determination as unconfirmed.

Estimation of the effect of neutropenia

Of the confirmed clinical bacteraemia, Table 2 shows the distribution by mean ANC recorded within a given study week. Of the 191 patient-weeks with confirmed clinical bacteraemia, 4 (2 due to *S. aureus*, 1 to other Gram-positive cocci, and 1 to Gram-positive rods) did not have ANC measurements in the same study week, leaving 187 (97.9%) for which the primary exposure could be ascertained.

In order to determine the association between clinical bacteraemia and neutropenia, trend tests were performed after applying linear scores to the ANC strata, adjusting the overall estimates by organism category to detect effect modification (Table 3). ANC strata were coded as follows: 1 (> 1000/ μ l), 2 (751–1000/ μ l), 3 (501–750/ μ l), 4 (251–500/ μ l), and 5 (0–250/ μ l). Thus, a fitted rate ratio > 1 indicates increasing rate of bacteraemia as the ANC level declined from a given stratum to the adjacent lower stratum. The sole clinical bacteraemia due to a Gram-negative coccus (*Neisseria meningitidis*) was included in the count of all clinical bacteraemic patient-weeks,

Table 4. Observed rates of clinical bacteraemia (per 100 patient-weeks) according to ANC stratum, by organism category: Exposure defined as mean ANC recorded in study week up to and including index date of bacteraemia

	All CB*	Poly	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	Other GNR	<i>S. aureus</i>	Other GPC	GPR	P-wks (%)‡
<i>n</i> (cb)†	181	19	15	10	22	31	58	49	11	26 779 (100)
ANC										
> 1000/ μ l	0.67	0.07	0.05	0.02	0.06	0.12	0.23	0.19	0.04	24 508 (91.5)
751–1000/ μ l	0.40	0.08	0	0	0.24	0.08	0.08	0.08	0.08	1250 (4.7)
501–750/ μ l	0.90	0	0.30	0.45	0.15	0	0	0	0	669 (2.5)
251–500/ μ l	1.18	0	0.39	0.39	0	0	0.39	0	0	255 (0.9)
0–250/ μ l	4.12	1.04	0	0	3.12	0	0	2.06	0	97 (0.4)

* Refer to note for Table 1 for summary of column abbreviations for organism categories.

† *n*(cb): number of clinical bacteraemic episodes of specified organism category with ANC exposure data available in same study week.

‡ Patient-weeks (% of total patient-weeks) of exposure in specified ANC stratum.

but the organism category of Gram negative-cocci was not included in stratified analyses of organism categories nor included in pooling across strata in Mantel–Haenszel analyses. Also, to avoid double counting of bacteraemic episodes, the polymicrobial organism stratum was not included in pooled analyses since each polymicrobial event was also represented in one of the other organism categories. Of the 187 patient-weeks having both confirmed clinical bacteraemia and ANC measurements at any time during the index study-week, six had no ANC measurements prior to or on the index date within the same study week. These six bacteraemic patient-weeks were distributed by organism category as: two polymicrobial bacteraemias (*K. pneumoniae* + other Gram-negative rod; *S. aureus* + other Gram-positive coccus), one due to *K. pneumoniae*, one to other Gram-negative rod, one to *St. aureus*, and one to other Gram-positive coccus. After excluding these six cases, the results of the analysis revealed a significant trend in effect for neutropenia defined as mean ANC. However, the effect of neutropenia varied substantially by organism category. The major effect of neutropenia is evident among gram negative rods including *E. coli*, *K. pneumoniae*, and *P. aeruginosa*.

In Table 4, the observed, rather than fitted, rates (per 100 patient weeks) of clinical bacteraemia are presented along with the person-time exposure within

each ANC stratum, overall and by organism category. To test the robustness of the observed relationships between neutropenia and clinical bacteraemia to the classification as *non-clinical bacteraemia* of potential bacteraemia for which medical records could not be located, the data were re-analysed after reclassifying unconfirmed bacteraemic weeks as true clinical bacteraemias. This analysis pertained to only a few potential cases (one polymicrobial, seven *S. aureus*, and 4 Gram-positive rod bacteraemic weeks). The single reclassified polymicrobial patient-week was associated with ANC mean in the > 1000/ μ l strata and the associated chi-square for trend remained non-significant ($P = 0.75$). Of the seven re-classified potential *S. aureus* patient-weeks, one had no ANC measures within the same week. Regarding mean ANC distribution of the remaining six *S. aureus* patient-weeks, five had mean ANCs in the > 1000/ μ l category and one in the 501–750/ μ l category. Trend tests for *S. aureus* remained non-significant for mean ANC exposure assignment rules ($P = 0.32$). All four of the reclassified potential Gram-positive rod bacteraemic weeks were associated with mean ANCs of > 1000/ μ l. The associated trend test was not significant ($P = 0.30$). Thus, the major conclusions reported in Tables 3 and 4 remain robust to re-classification of unconfirmed bacteraemias as true clinical bacteraemias.

Table 5. Assessment of intravascular catheter and GCSF use as a possible confounders of the association of neutropenia with clinical bacteraemia due to *E. coli*, *K. pneumoniae*, or *P. aeruginosa* ($n = 47$ cases)

	Percent of weeks with mean ANC < 500/ μ l		
	Bacteraemic weeks (%) ($n = 47$)	Non-bacteraemic weeks (%) ($n = 26738$)	Rate ratio*
Intravascular catheter			
All weeks	17	1.3	15.1
Catheter weeks	11.1	?	?
Non-catheter weeks	20.7		
GCSF			
All weeks	17	1.3	15.1
GCSF weeks	36.4	?	?
Non-GCSF weeks	11.1	?	?

* Unadjusted rate ratio for clinical bacteraemia due to *E. coli*, *K. pneumoniae*, or *P. aeruginosa*, comparing ANC strata (< 500 μ l/ \geq 500 μ l).

Because the effects of neutropenia were restricted to three Gram-negative rods, a further analysis was conducted to quantify the relative rates of bacteraemia due to any of the three organisms ($n = 47$ episodes) in more clinically meaningful terms. Taking ANC > 1000/ μ l as the reference category, the relative rates and 95% confidence intervals corresponding to lower ANC strata were: 751–1000/ μ l, 1.12 (0.27–4.66); 501–750/ μ l, 2.11 (0.51–8.76); 251–500/ μ l, 13.58 (5.32–34.65); 0–250/ μ l, 21.89 (6.73–71.17).

Exploratory analysis of confounding

Finally, to assess whether the unadjusted rate ratios for bacteraemia could be explained entirely by confounding due to the differential presence of central venous catheters and GCSF use, absolute neutrophil count was dichotomized into two strata (< 500/ μ l, \geq 500/ μ l), and the proportion of patient weeks with mean neutrophil counts < 500/ μ l was examined separately for bacteraemic and non-bacteraemic weeks. Table 5 shows separate analyses exploring the potential confounding effects of intravascular catheters and of GCSF on the association between neutropenia and bacteraemia due to *E. coli*, *K. pneumoniae*, or *P. aeruginosa*. The unadjusted rate ratio is 15.1 ($P < 0.001$). The prevalence of neutrophil counts < 500/ μ l for bacteraemic weeks with and without catheters and GCSF is substantially higher than the comparable prevalence for non-bacteraemic study weeks. This implies that the observed as-

sociation between neutropenia and gram negative rod bacteraemia cannot be explained entirely by differential distribution of catheters and GCSF by ANC strata.

DISCUSSION

In a historical cohort analysis involving over 26 000 patient-weeks of observation, a significant linear association was demonstrated between absolute neutrophil count categorized into five ordinal strata and risk of clinical bacteraemia occurring within the same calendar week. While the rate ratio estimates for bacteraemia due to any organism category were statistically significant in pooled analyses, the overall effect was small (RR < 2) and masked substantial heterogeneity by organism category. Clearly, the major consistent association of neutropenia was with bacteraemia due to three aerobic gram negative rods: *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. Although previous reports in the literature have not demonstrated this organism-specific association, these three organisms together with *S. aureus* have been the predominant bacterial pathogens associated with neutropenia attributable to cancer chemotherapy [18, 21].

The exposure measurement methodology used in this study was conservative in requiring neutrophil count to be known within a 7-day calendar interval. Other investigators have used broader time intervals within which neutrophil exposure information is

summarized. Moore and colleagues [15], for example, added 30 days both before and after an ANC dropped below designated thresholds in defining a 'neutropenic window' for each patient. Jacobson and colleagues [16] described a 'risk window' that could extend from 30 days before an ANC determination to 44 days after the measurement. By limiting extrapolation of neutrophil stratum assignment to 7-day intervals, we hoped to minimize exposure misclassification. One cost of this strategy was the loss of potential study-weeks at risk of bacteraemia. However, the size of the database was sufficient to compensate for this loss of information and still demonstrate the hypothesized association. A second cost was the inability to assess duration of neutropenia beyond 7 days, a factor previously demonstrated to augment risk for infectious complications associated with neutropenia (1). It is possible that additional associations would have emerged if, for example, we summarized ANC exposure information using 14 day intervals to define the neutropenic window. Nonetheless, for average neutrophil exposure within a given week, the absolute neutrophil measure demonstrated an ordinal 'dose-response' relationship to risk of the study outcome.

Because we had no control over the frequency of ascertainment of ANC levels across study weeks, biased estimates of association between neutropenia and risk of clinical bacteraemia could occur if the pattern of censoring of ANC information between weeks was related both to the ANC level in preceding weeks and to the study outcome. Separate analysis (not reported here) demonstrated a clear association between frequency of ANC ascertainment and preceding neutrophil levels, an observation to be expected based on reasonable clinical practice. That is, those who evidence lower ANC counts are likely to be monitored more frequently in subsequent weeks. On the other hand, because clinical bacteraemia is such a dramatic and symptomatic medical event, unless fatal immediately, bacteraemic patients will present for medical care and have both blood cultures and haematology measurements.

With regard to clinical bacteraemia, the design assumed that if patients became bacteraemic within a given study week, they would present to the study institution for care. It is perhaps reasonable to assume that since they had laboratory tests at the study institution during the same week, most would at least be likely to present for care in the event of bacteraemia. The proportion of outcomes lost by out of study institution care or death at home cannot be

directly assessed. We have no reason to believe, however, that such losses would be systematically related to the level of neutrophil count, thereby potentially biasing the observed associations. Misclassification of a potential bacteraemia as a clinical event is another potential source of bias in the study. The decision to classify a potential bacteraemia as a clinical event is not always clear cut, even when prospectively assessed [22–24]. In previous studies, organism type, days until the blood culture became positive, number of positive cultures, and clinical factors have contributed to multipredictor models for distinguishing clinical bacteraemia from contaminated cultures. We used medical record abstraction by study physicians to decide if a potential bacteraemia should be counted as a study outcome. Each of the abstraction forms was independently reviewed by three study physicians without knowledge of neutrophil data and an implicit judgement was made. Strengthening the results of the study was the uniformity of agreement among the record reviewers on outcome classification for the three aerobic Gram-negative enteric organisms for which strong associations with neutropenia were demonstrated. Assuming that any misclassification of study outcomes due to disagreement among the reviewers was non-differential with regard to the exposure variable neutropenia because of blinding, the effect on the results would be toward the null rate ratio [25]. That is, it would be harder to detect a true effect.

We have presented our results using rate ratios as measures of effect. Obviously, the clinical question of relevance is whether these parameters can be interpreted as indicating a causal relationship. While neutropenia has been established as a risk factor for infection, [1] in the case of bacteraemia due to Gram-negative enteric bacilli, release of endotoxin has been repeatedly shown to produce a transient dose related neutropenia as a result of cell margination and sequestration followed in a few hours by neutrophilia [26–28]. Given the limitations of our study design, we attempted to minimize temporal ambiguity by analysing the data after eliminating from consideration those neutrophil counts recorded within a given study week but after the index date of bacteraemia. The ideal situation would be to have daily measurements of neutrophil count for each of the 14 days prior to the index date of bacteraemia and to obtain blood cultures promptly at the first clinical sign or symptom of bacteraemia. Lacking this degree of experimental control, we cannot assert that a causal association has

been demonstrated. Nonetheless, the findings demonstrate a dose-response effect and are consistent with associations observed with neutropenia in other settings.

Exploratory analyses shown in Table 5, using methods illustrated by Ray and Griffin [20], demonstrated that the observed association of neutropenia with bacteraemia due to any of the three enteric Gram-negative organisms is unlikely to be explained by confounding due to the presence of intravascular catheters or G-CSF. A number of other potential confounding factors were not controlled for in this study: (1) integrity of mucosal surfaces, (2) recent injection drug use, (3) prior antibiotic use, (4) mechanism of neutropenia, and (5) CD4 count. In addition, the effects of neutropenia may be modified by duration of neutropenia and cytokine use. We are currently conducting a nested case-control study using the same study cohort to control for these factors.

From a clinical perspective, our analyses would suggest that, compared to patients with average ANC $> 1000/\mu\text{l}$, those with an average ANC of between $501\text{--}750/\mu\text{l}$ may experience doubling the baseline rate of gram negative enteric bacteraemia. As average ANC falls to $501\text{--}750/\mu\text{l}$, the associated relative rate is 13-fold higher, and for those with the lowest average ANC ($0\text{--}250/\mu\text{l}$), the rate is 22-fold higher than the reference category ($> 1000/\mu\text{l}$). If this association is confirmed after control for confounding factors, strong observational evidence of risk will have been demonstrated. In the meantime, the findings support a vigilant and preventive strategy in managing HIV infected patients with levels of neutropenia comparable to those known to confer risk in other populations.

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