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Influence of Referral Bias on the Clinical Characteristics of Patients with Gram-Negative Bloodstream Infection

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Summary

Referral bias can influence the results of studies performed at tertiary-care centers. In this study, we evaluated demographic and microbiologic factors that influenced referral of patients with gram-negative bloodstream infection (BSI). We identified 2919 and 846 unique patients with gram-negative BSI in a referral cohort of patients treated at Mayo Clinic Hospitals and a population-based cohort of Olmsted County, Minnesota, residents between 1/1/1998 and 12/31/2007, respectively. Multivariable logistic regression analysis was used to determine factors associated with referral. Elderly patients aged ≥ 80 years with gram-negative BSI were less likely to be referred than younger patients (odds ratio [OR]=0.43, 95% confidence intervals [CI]: 0.30-0.62) as were females (OR=0.63, 95% CI: 0.53-0.74). After adjusting for age and gender, bloodstream isolates of *Escherichia coli* (OR=0.50, 95% CI: 0.43-0.58) and *Proteus mirabilis* (OR=0.49, 95% CI: 0.30-0.82) were underrepresented in the referral cohort; and *Pseudomonas aeruginosa* (OR=2.26, 95% CI: 1.70-3.06), *Enterobacter cloacae* (OR=2.31, 95% CI: 1.53-3.66), *Serratia marcescens* (OR=2.34, 95% CI: 1.33-4.52) and *Stenotrophomonas maltophilia* (OR=17.94, 95% CI: 3.98-314.43) were overrepresented in the referral cohort. We demonstrated that demographic and microbiologic characteristics of patients with gram-negative BSI had an influence on referral patterns. These factors should be considered when interpreting results of investigations performed at tertiary-care centers.

Keywords

gram-negative; bacteremia; epidemiology; selection bias; population-based; Rochester Epidemiology Project

Introduction

Referral bias occurs as a result of systematic selection of patients from tertiary-care centers for inclusion in studies as the clinical features of patients presenting to tertiary-care referral centers with a particular illness differ from those in the community or general population

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[1]. Population-based studies lack referral bias as they include all patients with the disease of interest within a well-defined geographic area whether they present to primary, secondary or tertiary-care centers for medical care.

Only a few previous studies have evaluated the influence of referral bias on the clinical features of patients with infectious diseases, including infective endocarditis [2,3]. We have previously demonstrated the effect of referral bias on the *in vitro* antimicrobial resistance rates of *Pseudomonas aeruginosa* bloodstream isolates in an age- and gender-matched referral and population-based cohorts [4]. In the current study, we evaluated the influence of referral bias on the demographic and microbiologic characteristics of patients with gram-negative BSI by comparing a referral cohort of patients with gram-negative BSI to a population-based cohort within the same geographic area and time period. We aimed to (i) determine the influence of age and gender on referral patterns and (ii) examine the impact, if any, of the microbiologic etiology of gram-negative BSI on referral.

Materials and Methods

Settings

Olmsted County is located in southeastern Minnesota and has a population of 124,277 according to the 2000 census [5]. With the exception of a lower prevalence of persons who inject illegal drugs, a higher prevalence of middle-class persons and a higher proportion of persons employed in the healthcare industry, the population characteristics of Olmsted County residents are similar to those of USA non-Hispanic whites [2,6]. The Rochester Epidemiology Project (REP) is a unique medical records-linkage system that encompasses care delivered to residents of Olmsted County, Minnesota [6]. The microbiology laboratories at Mayo Medical Center and Olmsted Medical Center are the only two laboratories in Olmsted County. These two medical centers are geographically isolated from other urban centers as previously described [4,7], which increases the likelihood that residents get their healthcare at the local facilities, rather than seeking healthcare at a distant geographic location.

There are three hospitals in Olmsted County. The two Mayo Clinic-affiliated hospitals, St. Mary's and Rochester Methodist, are large tertiary-care centers that combine for over 1,950 licensed beds and provide care for both referral and local patients in a wide variety of medical and surgical subspecialties. The third hospital, Olmsted Medical Center, is a community-based hospital that provides primary care to local residents.

Case ascertainment

The population-based and referral cohorts where defined based on patient residency status, rather than the hospital where they received care. The population-based cohort consists of local (limited to Olmsted County residents) patients who were treated at any of the three hospitals. The referral cohort consists of patients who lived outside Olmsted County and received care at any of the three hospitals.

All residents of Olmsted County, Minnesota, were eligible for inclusion in the population-based cohort of the study as we used complete enumeration of the Olmsted County population from 1 January 1998 to 31 December 2007. After the institutional review boards of Mayo Medical Center (Rochester, Minnesota) and Olmsted Medical Center approved the study, we used the microbiology laboratory databases at both institutions to identify all episodes of gram-negative BSI during the study period. Using the REP tools, we identified residents of Olmsted County, Minnesota, for inclusion in the population-based cohort; patients living outside Olmsted County formed the referral cohort. The referral cohort could be either self-referred or physician-referred to Mayo Medical Center hospitals for management of gram-negative BSI. The primary investigator (M.N.A.) reviewed the medical records of all patients to confirm the diagnosis, determine patient residency status and obtain demographic and microbiologic features.

Case definition

Gram-negative BSI was defined as the growth of any aerobic gram-negative bacillus in a blood culture. Monomicrobial gram-negative BSI was defined as the growth of only one species of gram-negative bacillus in a blood culture. Coagulase-negative staphylococci, *Corynebacterium* spp. and *Propionibacterium* spp. were considered blood culture skin contaminants when isolated with gram-negative bacilli, and in cases where any of these were recovered, the BSI was not designated as polymicrobial.

The detailed blood culture methods used were described elsewhere [8,9]. Blood cultures were processed using standard microbiology techniques according to the Clinical and Laboratory Standards Institute (CLSI). The microbiology laboratories at the Mayo Medical Center Rochester and Olmsted Medical Center are certified by the College of American Pathologists.

Statistical analysis

Multivariable logistic regression analysis was used to examine the impact of age, gender and calendar year on referral patterns of patients with gram-negative BSI. Age was categorized into five groups (0-18, 19-39, 40-59, 60-79, and ≥ 80 years). Multivariable logistic regression analysis was also used to evaluate the effect of microbiologic etiology of gram-negative BSI on referral, after adjusting for age group and gender. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to indicate the strength of association with referral. JMP (version 8.0, SAS Institute Inc, Cary, NC) was used for statistical analysis. The level of significance for statistical testing was defined as $p<0.05$ (2-sided).

Results

We identified 846 Olmsted County residents with first episodes of monomicrobial gram-negative BSI between 1998 and 2007. The median age of patients in this population-based cohort was 68 years (interquartile range [IQR]: 47-81) and 56.5% were females.

During the same time period, 2919 patients with first episodes of monomicrobial gram-negative BSI living outside Olmsted County were treated at Mayo Medical Center hospitals in Rochester, MN. The median age of patients in this referral cohort was 63 years (IQR: 49-74) and 44.0% were females.

There was no detectable change in referral patterns over calendar years 1998 through 2007 (OR=0.99, 95% CI: 0.97-1.01 per year). Table 1 demonstrates the influence of age group and gender on referral of patients with gram-negative BSI. Compared to the reference age group of 0-18 years, elderly patients aged ≥ 80 years were less likely to be referred (OR=0.43, 95% CI: 0.30-0.62). Females with gram-negative BSI were also less likely to be referred than males (OR=0.63, 95% CI: 0.53-0.74).

Microbiology

Escherichia coli was the most common gram-negative bacillus that caused BSI in both cohorts, but it was underrepresented in referral patients (adjusted OR=0.50, 95% CI: 0.43-0.58). It contributed to over one-half (54.0%) of episodes of gram-negative BSI in the population-based cohort, but only 34.8% of episodes in the referral cohort (Table 2). *Proteus mirabilis* was also underrepresented in referral patients, even after adjusting for age group

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and gender (adjusted OR=0.49, 95% CI: 0.30-0.82). In contrast, *P. aeruginosa, Enterobacter cloacae, Serratia marcescens* and *Stenotrophomonas maltophilia* were overrepresented in the referral cohort. *S. maltophilia* was 18 times more likely to be reported as a cause of gram-negative BSI from a tertiary care referral center as compared to a population-based cohort. Despite being an extremely uncommon cause of gram-negative BSI in the general population contributing to only 0.1% of cases, it was the eighth most common gram-negative bacillus causing BSI in the referral cohort accounting for 2.3% of cases.

Discussion

The majority of studies of BSI published in the medical literature were performed at large tertiary-care centers; related population-based studies have been scant. The emphasis on institutional studies and rarity of population-based studies has provided a perception that the demographic, microbiologic and clinical characteristics of patients with gram-negative BSI treated at tertiary-care centers have been applicable to all settings. Institutional studies from tertiary-care centers systematically select patients with certain characteristics who are more likely to be referred for care at these centers. The differences in the results of institutional and population-based studies of gram-negative BSI have been mostly overlooked and often attributed to geographical and time variations. This study is the first to directly compare a referral and population-based cohort of patients with gram-negative BSI within the same geographic area and during the same period of time.

In this study, we demonstrated a difference in the demographic features of referral patients with gram-negative BSI and those in the general population. Elderly patients with gram-negative BSI were less likely to receive care at tertiary-care referral centers. This underrepresentation of elderly patients in studies performed at tertiary-care centers has been previously described, for example, in other infectious conditions, including infective endocarditis, and noninfectious conditions, such as Alzheimer's disease [2,10]. It is conceivable that elderly patients were less likely to be referred to tertiary-care centers due to patients and family wishes not to pursuit more aggressive medical care. It is also possible that elderly patients prefer to seek medical care in local primary- and secondary-care centers closer to home and family.

Females were also less likely to be referred for tertiary-care centers for management of gram-negative BSI. Female patients with gram-negative BSI are more likely than males to have a urinary tract primary source of infection [11]. It is conceivable that females with gram-negative BSI due to acute pyelonephritis were less likely to be referred to tertiary-care centers due to the less complex management requirements of their illness as compared to patients with other sources of infection, such as liver abscesses, for example, who might need surgical or interventional radiologic procedures that might be available only at large tertiary-care centers.

The microbiologic distribution of gram-negative bacilli that caused gram-negative BSI was also different between referral patients and those in the community. Despite the fact that *E. coli* was the most common pathogen causing BSI in several population-based studies from different continents [12-18], most institutional studies have reported that *Staphylococcus aureus* has been the most common cause of BSI [19-24]. This observation was also previously demonstrated locally. In a cross-sectional population-based study in Olmsted County, Minnesota, *E. coli* was the most common microorganism causing BSI contributing to 25% of cases followed by *S. aureus* (17%) [8]. On the other hand, in a previous institutional study including all episodes of BSI at Mayo Clinic (Rochester, Minnesota), *S. aureus* was the most common microorganism followed by *E. coli*, accounting for 17% and 12% of cases, respectively [25]. Our current study suggests that the underestimation of *E.*

coli as a cause of BSI in tertiary-care referral centers likely explains this phenomenon. It is not surprising that patients with *S. aureus* BSI were more likely to present for medical care at tertiary-care centers than those with *E. coli* BSI. *S. aureus* BSI is more likely than *E. coli* BSI to cause more serious complications such as infective endocarditis, epidural abscesses and deep surgical site infections that often require surgical interventions that may not be available at local primary- and secondary-care centers [26]. On the other hand, the urinary tract is predominantly the most common source of infection in patients with *E. coli* BSI.[11] Therefore, patients infected with this organism likely receive care at their local primary- and secondary-care centers, as transfers to tertiary-care centers for this indication may not be warranted in many cases. *P. mirabilis* was also underestimated in the referral cohort. This is also conceivable since *P. mirabilis* BSI, similar to *E. coli* BSI, is usually associated with a urinary tract primary source of infection [27].

The observation that *S. maltophilia* BSI was nearly 18-fold overestimated in the referral cohort, as compared to the population-based cohort of the study, is an excellent example of referral bias. This observation highlights the importance of clearly understanding the setting of where each study is performed prior to generalizing its results to other populations. *S. maltophilia* BSI is usually seen in cancer patients, especially those with hematogenous malignancies, neutropenia, central venous catheters, and those receiving broad-spectrum antimicrobial agents, particularly carbapenems [28-31]. Cancer is much more prevalent in hospitalized patients at large tertiary-care centers that provide care for cancer patients, such as Mayo Medical Center, than in the general population. Therefore, *S. maltophilia* is a much more common cause of gram-negative BSI in tertiary-care center series than in population-based studies.

Other gram-negative bacilli that are usually associated with nosocomial and healthcare-associated infections such as *P. aeruginosa, E. cloacae* and *S. marcescens* were also more common causes of BSI in tertiary-care centers than in the general population. Patients with BSI due to these microorganisms are more likely to have comorbid medical conditions requiring treatment at tertiary-care centers than those with *E. coli* and *Klebsiella pneumoniae* BSI, for example. In previous population-based studies, 78% and 92% of BSI episodes due to *P. aeruginosa* and *E. cloacae* were acquired in the hospital or healthcare setting [4,32], as compared to only 41% and 52% of episodes of *E. coli* and *K. pneumoniae* BSI, respectively [11,33].

The primary strengths of our study were the large sample size and the inclusion of patients with gram-negative BSI in both referral and population-based settings over a 10-year period of time.

Our study has limitations. First, the population of Olmsted County consists mainly of middle class whites; therefore, our study results may be generalized only to communities with similar population characteristics. Second, our data were derived from one geographic area. The results of studies from multiple geographic locations might provide a more generalizable view. Finally, detailed clinical variables were not collected in all patients and thus we were unable to compare underlying medical conditions, primary source of infection, and outcomes between the referral and population-based cohorts.

In summary, patients with gram-negative BSI presenting to tertiary-care centers have different demographic and microbiologic characteristics as compared to those in the general population. Gram-negative BSI surveys from tertiary-care centers tend to identify younger patients, males, and those with gram-negative microorganisms that cause nosocomial or healthcare-associated infections. The underestimation of *E. coli* BSI in tertiary-care center series resulted in differences in the most common cause of BSI in institutional studies versus population-based investigations. Physicians should be aware of the influence of referral bias on the results of institutional surveys performed at tertiary-care centers and should consider this before generalizing results to other settings.

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Table 1

Demographic characteristics of patients with gram-negative bloodstream infection in referral and population-based cohorts.

Variable	Referral cohort N=2919	Population-based cohort N=846	OR (95% CI)	P-value
Age group (in years)				<0.001
0-18	183 (6.3)	48 (5.7)	Reference	
19-39	260 (8.9)	98 (11.6)	0.71 (0.48-1.06)	
40-59	806 (27.6)	169 (20.0)	1.22 (0.85-1.75)	
60-79	1267 (43.4)	290 (34.3)	1.08 (0.77-1.53)	
≥ 80	403 (13.8)	241 (28.5)	0.43 (0.30-0.62)	
Gender				<0.001
Male	1635 (56.0)	368 (43.5)	Reference	
Female	1284 (44.0)	478 (56.5)	0.63 (0.53-0.74)	

OR: odds ratio, CI: confidence interval.

Data are given as number (%) unless otherwise specified.

Table 2

Distribution of the ten most common pathogens causing gram-negative bloodstream infection in the referral and population-based cohorts.

Pathogen	Referral cohort N=2919	Population-based cohort N=846	Unadjusted OR (95% CI)	Adjusted OR* (95% CI)
Escherichia coli	1015 (34.8)	457 (54.0)	0.45 (0.39-0.53)	0.50 (0.43-0.58)
Klebsiella pneumoniae	403 (13.8)	102 (12.1)	1.17 (0.93-1.47)	1.18 (0.93-1.49)
Pseudomonas aeruginosa	419 (14.4)	55 (6.5)	2.41 (1.80-3.23)	2.26 (1.70-3.06)
Enterobacter cloacae	190 (6.5)	24 (2.8)	2.38 (1.55-3.67)	2.31 (1.53-3.66)
Acinetobacter species	114 (3.9)	20 (2.5)	1.68 (1.04-2.72)	1.52 (0.96-2.54)
Serratia marcescens	102 (3.5)	12 (1.4)	2.52 (1.38-4.60)	2.34 (1.33-4.52)
Klebsiella oxytoca	98 (3.4)	18 (2.1)	1.60 (0.99-2.74)	1.58 (0.97-2.71)
Stenotrophomonas maltophilia	67 (2.3)	1 (0.1)	19.74 (4.38-345.67)	17.94 (3.98-314.43)
Enterobacter aerogenes	51 (1.7)	10 (1.2)	1.49 (0.79-3.21)	1.40 (0.74-2.95)
Proteus mirabilis	41 (1.4)	25 (3.0)	0.47 (0.28-0.77)	0.49 (0.30-0.82)

OR: odds ratio, CI: confidence interval.

Data are given as number (%) unless otherwise specified.

*Odds ratios in this column are adjusted for age group and gender.