Significance of Mannose-Binding Lectin Deficiency and Nucleotide-Binding Oligomerization Domain 2 Polymorphisms in *Staphylococcus aureus* Bloodstream Infections: A Case-Control Study

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Abstract

**Background:** Pathways coordinated by innate pattern recognition receptors like mannose-binding lectin (MBL) and nucleotide-binding oligomerization domain 2 (NOD2) are among the first immune responses to *Staphylococcus aureus* (*S. aureus*) bloodstream infections (BSI) in animal models, but human data are limited. Here, we investigated the role of MBL deficiency and NOD2 mutations in the predisposition to and severity of *S. aureus* BSI.

**Patients and Methods:** A matched case-control study was undertaken involving 70 patients with *S. aureus* BSI and 70 age- and sex-matched hospitalized controls. MBL levels, *MBL2* and *NOD2* polymorphisms were analyzed.

**Results:** After adjusting for potential confounders, MBL deficiency (<0.5 µg/ml) was found less frequently in cases than controls (26 vs. 41%, OR 0.4, 95% confidence interval (CI) 0.20-0.95, p=0.04) as were low producing MBL genotypes (11 vs. 23%, OR 0.2, 95% CI 0.08-0.75, p=0.01), whereas *NOD2* polymorphisms were similarly distributed. Cases with *NOD2* polymorphisms had less organ dysfunction as shown by a lower SOFA score (median 2.5 vs. 4.5, p=0.02), whereas only severe MBL deficiency (<0.1 µg/ml) was associated with life-threatening *S. aureus* BSI (OR 5.6, 95% CI 1.25-24.85, p=0.02).

**Conclusions:** Contrary to animal model data, our study suggests MBL deficiency may confer protection against acquiring *S. aureus* BSI. *NOD2* mutations were less frequently associated with multi-organ dysfunction. Further human studies of the innate immune response in *S. aureus* BSI are needed to identify suitable host targets in sepsis treatment.

Introduction

*Staphylococcus aureus* (*S. aureus*) is a major cause of nosocomial and community-acquired bloodstream infections (BSI) accounting for up to 20% of hospital isolates [1]. *S. aureus* BSI is associated with a high morbidity and mortality compared to other BSI pathogens [2] and when it is caused by methicillin resistant isolates the mortality is even greater [3]. These infections place a huge burden on health care systems due to a longer duration of hospital stay and higher total treatment cost compared to bacteremia caused by any other pathogen [4]. In addition, the incidence of *S. aureus* BSI has steadily increased over the past 30 years as a consequence of frequent use of intravascular devices and invasive procedures [5]. General host risk factors for the acquisition of *S. aureus* BSI include staphylococcal colonization, surgical site infection, injection drug use, presence of immunosuppressive conditions and liver disease [2]. Central to the pathogenicity and immune evasion of *S. aureus* is the coordinated activity of several virulence factors including surface-expressed adhesins, complement inhibitors, exotoxins and exoenzymes that facilitate direct tissue destruction while avoiding activation of the innate immune system, particularly the complement system [6]. However, human studies examining the impact of the
innate immune system on the susceptibility to and the severity of S. aureus BSI are limited [7,8].

Pattern recognition receptors (PRR) are crucially involved in the initial and immediate immune response against S. aureus (reviewed in [9]). In particular, nucleotide-binding oligomerization domain 2 (NOD2) and mannose-binding lectin (MBL) have been implicated in the pathogenesis of S. aureus infections in several experimental models. NOD2 is an intracellular sensor for both gram-positive and -negative bacterial cell wall components leading to a pro-inflammatory NF-κB and IL-1β mediated cytokine response (reviewed in [10]), although the exact mechanism and regulation of response in bacterial infections still remain to be fully elucidated. Animal model data on S. aureus and NOD2 are conflicting [11–13]. Results from two studies involving critically-ill sepsis patients suggest an increased risk of bacteremia and mortality in individuals with at least one NOD2 variant [14,15].

MBL, a liver-derived circulating lectin contributes to the efficient removal of pathogens and apoptotic cells by activating the lectin pathway of complement and promoting opsonophagocytosis [16], and has been implicated as an important defense mechanism in various infectious diseases [17]. Functional MBL deficiency is common in humans and is caused by polymorphisms within the coding and promoter regions of the MBL2 gene on chromosome 10 [16]. In vitro, MBL is able to bind to S. aureus [19] and evidence from animal models suggests that MBL deficiency significantly increases the susceptibility to and severity of S. aureus bacteremia [20,21]. However, its contribution to S. aureus induced complement activation and phagocytosis of S. aureus in adults is probably less than the antibody-mediated classical pathway activation [22–24]. Several clinical studies have reported a correlation between MBL deficiency and increased susceptibility to bacterial sepsis in children and adults [25–27].

Given these data on the potential role of NOD2 and MBL in human innate immune defences against severe S. aureus infection we hypothesized that MBL deficiency and NOD2 mutations might be associated with increased susceptibility to and severity of S. aureus BSI.

Patients and Methods

Ethics statement
The study had been approved by the Melbourne Health Human Research and Ethics Committee and all participants gave written informed consent for the study.

Participants
We conducted a matched prospective case-control study at two major tertiary hospitals involving 70 patients with S. aureus BSI and 70 age- and sex-matched hospitalized controls. Investigators were notified of all blood cultures positive for S. aureus by the central microbiology laboratory during the study period (September 2009 to September 2011). Case patients were enrolled with their first S. aureus BSI if they were >18 years old and had at least 1 positive blood culture for S. aureus. Hospitalised control patients were selected on the absence of infection as the cause for admission and were matched for age (within 2 years) and sex. Controls had to be admitted within 2 months of the case patient. To increase the power for the analysis of severity after S. aureus BSI, 30 patients with S. aureus BSI with similar epidemiology from a previous study were included only in this component of the study [25]. MBL levels and MBL2 genotype have been previously reported for these patients, and demographic and clinical data similar to the patients recruited in this study was available. We were able to use stored genomic DNA samples from these patients to determine NOD2 polymorphisms.

Risk factors for staphylococcal BSI
Demographic, clinical and microbiological data were collected by investigators blinded to MBL and/or NOD2 results including comorbidities and presence of intravenous (IV) lines or urinary catheters before the episode of S. aureus BSI. Liver disease was defined as cirrhosis, chronic hepatitis B and C, hepatocellular carcinoma or any other significant acute or chronic liver disease. Renal disease included acute and chronic renal impairment of various reasons excluding hemodialysis. Patients were regarded as immunosuppressed if they were receiving chemotherapy, corticosteroids (≥7.5mg prednisolone equivalent per day), methotrexate, cyclosporine, tacrolimus, azathioprine or biologics such as TNF-α inhibitors.

The Sequential Organ Failure Assessment (SOFA) score was calculated for case patients on the day when the first positive blood culture was taken. A SOFA score of >7 was regarded as very severe disease being the mean score of non-survivors in the validation study of this score [28].

Determination of MBL plasma levels
EDTA blood samples which had been taken one to three days prior to the diagnosis of S. aureus BSI were accessed for further testing. Quantification of MBL plasma levels was performed by an investigator blinded to any patient data using a mannann-binding enzyme-linked immunosorbent assay as previously described [25,29]. Briefly, mannann-coated microtitre plates were incubated with samples at 1:25 and 1:100 dilutions for 90 min at room temperature followed by detection of bound MBL with a biotinylated monoclonal anti-MBL antibody (HYB 131-01, BioPorto Diagnostics, Denmark). MBL deficiency was defined as serum level < 0.5 μg/ml and, severe as < 0.1 μg/ml, respectively.

MBL2 and NOD2 genotyping
MBL2 promoter and first exon and NOD2 polymorphisms were determined by allele specific polymerase chain reaction (PCR) using TaqMan fluorescent probes (TaqMan genotyping assays, Life Technologies, Australia). For assay details, see Table S1. DNA lysates were prepared from 2µl of stored buffy coat according to the manufacturer’s instruction (TaqMan Sample-to-SNP, Life Technologies, Australia), and stored genomic DNA was used for 30 patients included in a previous study [25]. For all TaqMan assays, DNA amplification was carried out in 5µL volume reactions containing 1µl of DNA lysate or 20ng of genomic DNA, 0.25µl TaqMan genotyping assay mix, 2.5µl TaqMan GTXpress Master Mix (Life Technologies, Australia) and 1.25μl DNase-free water. All
reactions were performed in 384-well plates and in the ViiA 7 thermocycler (Life Technologies, Australia) according to the manufacturer's instructions. For allelic discrimination end-point fluorescence was read at 25°C, and the ViiA 7 software was used to analyze the results (Life Technologies, Australia).

**MBL2** genotypes were classified as low (XA/YO, YO/YO), intermediate (XA/XA, YA/YO) or high (YA/YA, XA/YA) producing genotypes according to published literature [26] with exon variant alleles collectively designated as O and the wild-type gene as A, and the promoter variant allele and the wild-type gene designated as X and Y, respectively.

**Definition of aims**

The main aim of this study used to determine the sample size, was to compare the frequency of MBL deficiency in patients with **S. aureus** BSI with age/sex-matched, hospitalized control patients. We recruited 70 cases and controls in order to have an 80% chance of detecting an odds ratio of 3, with an expected frequency of MBL deficiency (defined as plasma concentration <0.5 µg/ml) in the control population of 24% [29] at the 5% level of significance. Additional aims included measuring the effect of **NOD2** mutations on the risk of acquiring **S. aureus** BSI in cases compared to controls, and in cases alone, the influence of MBL levels and **MBL2** and **NOD2** mutations on the severity of **S. aureus** BSI as evaluated by the SOFA score and crude in-hospital mortality.

**Statistical analysis**

To investigate potential risk factors for acquiring **S. aureus** BSI, matched univariate analysis was performed by running conditional logistic regression on one variable at a time with **S. aureus** BSI as the dependent variable. In addition, Wilcoxon signed-rank test was applied to compare MBL levels in cases and matched controls. Multivariate conditional logistic regression models were used to estimate the effect of MBL deficiency on the risk of acquiring **S. aureus** BSI while adjusting for covariates with univariate p values less than 0.1 and which have been described in previous studies.

Regarding the severity of **S. aureus** BSI differences in outcome measures of cases according to patient characteristics, MBL levels and **MBL2** or **NOD2** mutations were first analyzed using the Fisher's exact, the χ² or the Mann-Whitney-U-Test where appropriate. Subsequently, stepwise binary logistic regression models were calculated to estimate the association of MBL levels and **MBL2** or **NOD2** mutations with predefined endpoints in multivariate analyses after adjustment for covariables with univariate p values less than 0.1. The Hardy-Weinberg equilibrium for **MBL2** and **NOD2** genotype frequencies was assessed by χ² statistics. All testing was two-tailed. All analyses were performed using SPSS statistics, version 17.0 (SPSS Inc., USA).

### Table 1. Clinical characteristics of **S. aureus** BSI cases and controls.

| Infective source                | Cases (n=70) | Controls (n=70) |
|---------------------------------|-------------|-----------------|
| Intravascular line              | 16 (23)     | 11 (16)         |
| Skin or soft tissue             | 11 (16)     | 14 (20)         |
| Bone or joint                   | 13 (19)     | 11 (16)         |
| Endocarditis                    | 7 (10)      | 5 (7)           |
| Pneumonia                       | 8 (11)      | 7 (10)          |
| Hemodialysis associated         | 11 (16)     | 8 (11)          |
| No source identified            | 4 (6)       |                 |

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|                     | Cases (n=70) | Controls (n=70) |
|---------------------|-------------|-----------------|
| PSSA                | 15 (21)     |                 |
| MRSA                | 12 (17)     |                 |

**Admission diagnosis, n (%)**

|                     | Cases (n=70) | Controls (n=70) |
|---------------------|-------------|-----------------|
| Trauma              | 18 (26)     |                 |
| Medical condition    | 29 (41)     |                 |
| Surgery (not trauma related) | 23 (33) |         |

**Abbreviations:** BSI, bloodstream infection; MRSA, methicillin-resistant **S. aureus**; PSSA, penicillin-sensitive **S. aureus**; **S. aureus**, Staphylococcus aureus.

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### Results

**Demographic and clinical characteristics of cases and controls**

The analyzed study population consisted of 70 **S. aureus** BSI cases and 70 age- and sex-matched, hospitalized controls. **S. aureus** BSI were nosocomially acquired (60%) and related to endovascular sources (49%) in the majority of cases. Controls were mainly admitted for trauma or elective surgery (Table 1). A median of 2 blood culture bottles were positive for **S. aureus**, and cultured isolates were methicillin resistant in 12/70 (17%) of cases (2/12 community-acquired). Antibiotic therapy appropriate for the susceptibility of the infective organism was received within 24 hours after blood cultures had been drawn in 59/70 (84%) cases.

In terms of risk factors, **S. aureus** BSI cases were more likely to suffer from liver disease, to require hemodialysis and to have long-term IV lines when compared to controls (Table 2). In contrast, controls were more likely to have undergone recent surgery, with peripheral IV lines and urinary catheters used more frequently at the time of recruitment. There was no difference in terms of prevalence of diabetes mellitus, heart disease, cancer or immunosuppression.

**Association of MBL and NOD2 variants with the risk of acquiring **S. aureus** BSI**

**MBL2** and **NOD2** allele frequencies at all 7 positions were in agreement with the predicted Hardy-Weinberg equilibrium (data not shown). There was significant correlation between **MBL2** genotypes and MBL levels (Kruskal-Wallis test, p<0.001,
data not shown). As expected, patients with low producing genotypes all had MBL levels <0.5 µg/ml. The frequency distribution of MBL2 genotypes differed significantly among cases and controls with higher number of intermediate and low genotypes in controls (Table 3). In line with literature and likely related to selection bias in the control group, α-melanocortin deficiency defined a priori hypothesis, MBL deficiency as defined by low producing genotypes or lower MBL plasma levels (p<0.001, Figure 1), and 18/70 of cases vs. 29/70 of controls (0.9 (IQR 0.2-2.4) vs. 2.7 (IQR 0.5-4.6) cases and controls with higher number of intermediate and low genotypes, p<0.001, Table 3). In a multivariate analysis (OR 6.01, 95% CI 1.70-21.54, p<0.01).

**Table 2. Analysis of clinical characteristics as predisposing risk factors for S. aureus BSI.**

| Variables                                      | Controls (n=70) | Cases (n=70) | Univariate matched analysis (OR 95% CI) P value* |
|------------------------------------------------|----------------|--------------|--------------------------------------------------|
| Age, mean (SD)                                 | 61 (17)        | 61 (18)      | 1.09 (0.9-1.32) p=0.4                           |
| Male sex, n (%)                                | 49 (70)        | 51 (73)      | 0.01-5x10^6 (0.45)                               |
| Diabetes, n (%)                                | 15 (21)        | 20 (29)      | 1.63 (0.67-3.92) p=0.28                         |
| Heart disease, n (%)                           | 36 (51)        | 30 (43)      | 0.46 (0.16-1.31) p=0.14                         |
| Cancer, n (%)                                  | 10 (14)        | 13 (19)      | 1.36 (0.55-3.42) p=0.5                          |
| Immunosuppressive treatment                   | 8 (11)         | 15 (21)      | 2.2 (0.82-5.70) p=0.12                          |
| Liver disease, n (%)                           | 0 (0)          | 15 (21)      | 65 (1.03-4148.5) p<0.05                         |
| Kidney disease, n (%)                          | 6 (9)          | 10 (14)      | 2.0 (0.80-6.64) p=0.26                          |
| Hemodialysis, n (%)                            | 3 (4)          | 16 (23)      | 5.33 (1.55-18.3) p<0.01                         |
| Iilit IV drug use, n (%)                       | 2 (3)          | 6 (9)        | 65 (0.02-2x10^5) 0.31                           |
| Recent surgery, n (%)                          | 41 (59)        | 7 (10)       | 0.03 (0.03-0.26) p<0.001                         |
| Urinary catheter, n (%)                        | 30 (43)        | 9 (13)       | 0.22 (0.09-0.54) 0.001                           |
| Vascular lines, n (%)                          | 14 (20)        | 28 (40)      | 2.1 (1.1-4.0) p=0.03                            |
| Long-term IV line                              | 57 (81)        | 26 (37)      | 0.11 (0.04-0.32) p<0.001                         |

**Table 3. Analysis of MBL2 and NOD2 genotypes in S. aureus BSI cases and controls.**

| Variables                                      | Controls (n=70) | Cases (n=70) | Univariate matched analysis (OR 95% CI) P value* |
|------------------------------------------------|----------------|--------------|--------------------------------------------------|
| MBL2 exon variants, n (%)                      | 30 (43)        | 44 (63)      | Reference                                        |
| A/A                                            | 22 (16)        | 1            | 0.24                                             |
| A/C                                            | 5 (1)          | 65 (1.03-4148.5) p<0.05 |
| A/D                                            | 8 (5)          | 5            | 0.44 (0.22-0.90) 0.024                          |
| Total A/O                                      | 35 (50)        | 22 (31)      | 0.4 (0.20-1.12) 0.09                            |
| BM2 promoter variants, n (%)                   | 42 (60)        | 52 (74)      | Reference                                        |
| Y/Y                                            | 13 (30)        | 1            | 0.47 (0.20-1.12) 0.27                            |
| X/X                                            | 14 (13)        | 1            | 0.27 (0.03-2.70) 0.27                            |
| Total high producing                           | 27 (39)        | 43 (61)      | Reference                                        |
| Reference                                      | 3 (4)          | 18 (22)      | 0.43 (0.20-0.94) 0.034                          |
| Intermediate producing                         | 27 (39)        | 19 (27)      | 0.43 (0.20-0.94) 0.034                          |
| Reference                                      | 11 (4)         | 4            | 0.31 (0.11-0.84) 0.021                           |
| Total low producing                            | 16 (23)        | 8 (11)       | 0.31 (0.11-0.84) 0.021                           |
| MBL levels (µg/ml), median (IQR)               | 0.9 (0.2-2.4)  | 2.7 (0.5-4.6) | 1.32 (1.1-1.58) 0.002                           |
| MBL <0.5µg/ml, n (%)                           | 29 (41)        | 18 (26)      | 0.50 (0.24-1.03) 0.06                            |
| NOD2 mutations, n (%)                          | 7 (10)         | 10 (14)      | 1.5 (0.53-4.21) 0.44                            |
| R702W C>T                                      | 4 (4)          | 2            | 0.27 (0.03-2.70) 0.27                            |
| G908R G>C                                      | 2 (2)          | 1            | 0.31 (0.11-0.84) 0.021                           |

**Table 3. Analysis of MBL2 and NOD2 genotypes in S. aureus BSI cases and controls.**

Abbreviations: BSI, bloodstream infection; CI, confidence interval; IQR, interquartile range; IV, intravenous; OD, odds ratio; SD, standard deviation; S. aureus, Staphylococcus aureus. Y and A denote promoter and exon wildtype alleles, respectively.

* univariate conditional logistic regression analysis.

by MBL levels <0.5 µg/ml (OR 0.44, 95% confidence interval (CI) 0.20-0.95, p=0.04) or low producing genotypes (OR 0.24, 95% CI 0.08-0.75, p=0.01) remained independently associated with a decreased risk of acquiring a S. aureus BSI. Otherwise, only hemodialysis was an independent predictor of S. aureus BSI in a multivariate analysis (OR 6.01, 95% CI 1.70-21.54, p<0.01).
Association of MBL and NOD2 variants with severity of *S. aureus BSI*

One hundred cases were analyzed for associations between MBL2 or NOD2 mutations and severity of *S. aureus* BSI. Clinical characteristics are displayed in Table 4. Regarding severity of *S. aureus* BSI the median SOFA score was 4 (IQR 2-6) and in-hospital mortality was 10% overall.

Organ dysfunction was less pronounced in patients with NOD2 mutations indicated by significantly lower SOFA scores (median 2.5 (IQR 0-4.1) vs. 4.0 (IQR 2-6), p=0.02) and the fact that the majority (8/16 (50%)) demonstrated a SOFA score of less than 3 and only 1/16 (6%) patient with a SOFA score >7 compared to 24/84 (29%) and 15/84 (18%) patients with NOD2 wild-type genotype, respectively (p=0.19). MBL deficiency (<0.5 µg/ml) had no influence on the severity overall as evaluated by the SOFA score (data not shown). However, severe MBL deficiency (<0.1 µg/ml) significantly increased the odds of a patient having severe organ dysfunction as defined by a SOFA score >7 (OR 5.57, 95% CI 1.25-24.85, p=0.02) after adjusting for age and gender. A similar non-significant trend was observed regarding severe MBL deficiency or NOD2 mutations and frequency of admission to ICU (4/10 (40%) vs. 18/90 (20%) and 2/16 (13%) vs. 20-84 (24%), respectively), whereas in-hospital mortality was not different.

**Discussion**

MBL and NOD2, two PRR of the innate immune system, have been implicated in the pathogenesis of *S. aureus* BSI in several experimental models [11–13,20,21]. This is the first human study designed to examine the effect of these two important first-line defense mechanisms on predisposition and severity of infection in *S. aureus* BSI patients, exclusively.

Despite previous experimental studies that were the basis for our *a priori* hypotheses, we did not demonstrate that MBL deficiency or NOD2 mutations predispose to *S. aureus* BSI. Interestingly, we found that rather the opposite is true in terms of MBL deficiency. MBL deficiency was associated with less than half the risk of acquiring *S. aureus* BSI. Previous studies

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**Figure 1. Plasma mannose-binding lectin levels in *S. aureus* BSI cases and controls.** Differences in plasma mannose-binding lectin levels in *S. aureus* BSI cases and controls. Short horizontal lines (whiskers) represent minimum and maximum levels whereas horizontal lines inside the box-plot represent medians. Abbreviations: BSI, bloodstream infection; MBL, mannose-binding lectin. *S. aureus*, *Staphylococcus aureus*. doi: 10.1371/journal.pone.0076218.g001

**Table 4. Clinical characteristics and outcomes in *S. aureus* BSI cases (n=100).**

| Variables                          | S. aureus BSI (n=100) | SOFA score |
|-----------------------------------|-----------------------|------------|
|                                   | <2 (n=32) | 3-7 (n=52) | >7 (n=16)  | P value  |
| Age, mean (SD)                    | 60.7 (18.7) | 63.7 (18.5) | 59.5 (19.0) | 58.5 (18.8) | 0.54  |
| Male sex, n (%)                   | 72 | 26 (81) | 38 (73) | 8 (50) | 0.07  |
| MBL2 genotypes, n (%)             |                     |            |            |            |      |
| high (YA/YA, XA/YA)               | 60 | 18 (56) | 33 (64) | 9 (56) | 0.84  |
| intermediate (YA/YO, XA/XA)       | 29 | 9 (28) | 15 (29) | 5 (31) |        |
| low (YO/YO, XA/YO)                | 11 | 5 (16) | 4 (8) | 2 (13) |        |
| MBL levels (µg/ml), median (IQR)  | 2.4 (0.5-4.1) | 3.0 (0.7-6.0) | 2.4 (0.5-3.6) | 1.0 (0.1-4.0) | 0.38  |
| MBL <0.5µg/ml, n (%)              | 25 | 7 (22) | 13 (25) | 5 (31) | 0.78  |
| MBL <0.1µg/ml, n (%)              | 10 | 4 (13) | 2 (4) | 4 (25) | 0.04  |
| NOD2 mutation, n (%)              | 16 | 8 (25) | 7 (14) | 1 (6) | 0.19  |
| Outcomes                          |                     |            |            |            |      |
| SOFA score, median (IQR)          | 4 (2-6) |            |            |            |        |
| ICU admission, n (%)               | 22 | 1 (3) | 11 (21) | 10 (63) | <0.001 |
| In-hospital mortality, n (%)       | 10 | 2 (6) | 5 (10) | 3 (19) | 0.4   |

Abbreviations: BSI, bloodstream infection; CI, confidence interval; ICU, intensive care unit; IQR, interquartile range; MBL, mannose-binding lectin; NOD2, nucleotide-binding oligomerization domain 2; OD, odds ratio; SD, standard deviation; S. aureus, *Staphylococcus aureus*; SOFA, sequential organ failure assessment; Y and A denote MBL2 promoter and exon wild type alleles, respectively.

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which failed to demonstrate an effect of MBL deficiency [25,30] were likely underpowered as they had only examined a limited number of S. aureus BSI patients as part of larger sepsis trials, and controls were not matched. Recent data may help to resolve this apparent contradiction. It has been demonstrated that wildtype MBL2 genotypes are associated with persistent S. aureus nasal carriage in adults, a well known predictor for subsequent invasive disease [31]. Additionally, studies suggest that binding of MBL to S. aureus might be restricted to infancy due to inhibitory anti-wall teichoic acid antibodies in adults [23] and subsequently that anti-staphylococcal complement activation and opsonophagocytosis is dominated by the C1q-dependent classical pathway independent of MBL [22]. Finally, data that suggest a non-redundant role of MBL in staphylococcal infections in infancy come from two recent clinical studies that show that infants with MBL2 mutations are more susceptible to S. aureus colonization [32] and fatal invasive methicillin-resistant S. aureus co-infections after influenza [33].

It is unlikely that an acute phase elevation of MBL accounts for the higher levels in cases as the degree of elevation is usually mild and restricted to wild-type patients [34], and more importantly, genotypic data of our case patients were consistent with MBL levels demonstrating high producing haplotypes in a significantly greater proportion compared to controls (61 vs. 39%).

We also found no difference in the prevalence of NOD2 mutations in S. aureus BSI cases and controls with the frequency and presence of only heterozygous mutations being in line with previous reports from an Australian control population [35]. This finding is consistent with the clinical observation (and preliminary evidence [36]) that (untreated) Crohn’s disease patients, who have a higher prevalence of NOD2 mutations than healthy controls, are not at an increased risk of S. aureus BSI.

Overall then it seems that physical factors (hemodialysis or intravenous catheters) or comorbidities (liver failure) account more for susceptibility to S. aureus BSI than genetic defects in the innate immune proteins we studies, at least in an adult population.

Although MBL deficiency or NOD2 mutations had no significant impact on mortality, we could demonstrate important associations with our other a priori measure, the severity of S. aureus BSI as evaluated by the SOFA score. Interestingly, patients with NOD2 mutations had significantly lower SOFA scores and admissions to ICU with 50% showing a SOFA score <3 as compared to only 29% of patients lacking tested NOD2 polymorphisms. Less pulmonary inflammation and faster recovery has been shown in NOD2 knockout mice during S. aureus pneumonia [13]. Similarly, a diminished initial inflammatory response was demonstrated in NOD2 knockout mice after subcutaneous challenge with S. aureus [12] although the mice developed significantly larger ulcerations later on possibly related to an impaired bacterial clearance. In contrast, NOD2 knockout mice were more susceptible to S. aureus infection in a peritoneal challenge model [11]. Currently available data on human sepsis associations with NOD2 mutations indicate more prevalent bacteremia and higher sepsis-related mortality in ICU studies [14,15]. However, both human studies included only a minority of patients with S. aureus BSI, hence the ability to compare with our study is limited. In theory, heterozygous NOD2 mutations might impair the recognition of S. aureus to a limited degree but also attenuate the initial excessive and dysfunctional inflammatory response [37,38]. In summary, this might effectively result in less host damage overall in S. aureus BSI assuming removal of the pathogen by timely administration of effective antibiotic treatment.

Only severe MBL deficiency (<0.1 µg/ml) was associated with critical disease as evaluated by the SOFA score and admission to ICU, which is in line with knockout animal models [20,21] and previous sepsis studies including a variety of infections [25,26,39]. However, the significance of this observation is limited by the small sample size of patients with severe MBL deficiency.

Our study has some limitations including the fact that microbial virulence factors shown to influence the severity of community-acquired invasive S. aureus infections recently [40] were not examined. In addition, data on S. aureus colonization rate, a recognized risk factor for invasive infections were not available in cases and controls. Severity according to the SOFA score was only evaluated once on the day the first positive blood culture was drawn before antibiotic therapy was initiated. However, this approach eliminates possible confounders introduced later by differences in treatment (e.g. antibiotic management, timing of surgical intervention or infectious diseases consultation). We limited our analysis of the innate immune system to two key PRR, which have been shown to be significantly involved in S. aureus infection, previously. Ideally, future studies should include other important PRR like TLR-2 [41] which are also involved in the pathogenesis of S. aureus infections. Although our analysis of the importance of MBL and NOD2 in S. aureus BSI is the largest to date, its significance is limited in terms of mortality due to low event numbers.

In conclusion, this study does not support an important role for either MBL or NOD2 in protecting adults from acquiring S. aureus BSI. In fact, contrary to previous animal model data our results show that MBL deficiency seems to confer significant protection from S. aureus BSI. In addition, heterozygous NOD2 polymorphisms were less frequently associated with organ dysfunction in S. aureus BSI consistent with the notion that outcomes of infections are more driven by the host response to microorganisms than by their direct toxic effects [37,38]. Our present state of knowledge indicates that possible effects of innate immune system abnormalities are likely overwhelmed by conventional risks factors for staphylococcal BSI.

**Supporting Information**

Table S1. Taqman genotyping assay details (Life Technologies, Australia).

(DOC)
Author Contributions
Conceived and designed the experiments: MO HMAY MMD DPE. Performed the experiments: MO HMAY MMD. Analyzed the data: MO, MMD DPE. Contributed reagents/materials/

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