

Title

Preserved C-reactive protein responses to blood stream infections following tocilizumab treatment for COVID-19

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Running title

CRP responses in tocilizumab-treated COVID-19

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1 Dear Editor,

2 In this Journal, Rossotti and colleagues provided early data on tocilizumab utility in COVID-19¹, later
3 confirmed in randomised studies². Tocilizumab-mediated inhibition of IL-6 signalling can decrease CRP
4 concentrations¹, potentially confounding the diagnosis of bacterial co-infections in COVID-19 that occur more
5 frequently following longer hospital stays and admissions to the intensive care unit (ICU)³⁻⁵.

6 In inflammatory arthritides, multiple tocilizumab dosing variably attenuates CRP responses following
7 bacterial infections⁶, but the effect following single-dose use in COVID-19 is not defined^{2,7}. In a small COVID-
8 19 cohort with blood stream infections (BSIs) that had received tocilizumab, CRP was reduced but remained
9 detectable at the time of BSI diagnosis⁸. However, CRP kinetics related to BSI were not assessed, and thus
10 the utility of CRP to guide antibiotic prescribing in this context remains unknown^{5,9}. We addressed this
11 question by testing the hypothesis that a single dose of tocilizumab for COVID-19 retained CRP responses to
12 bacterial infections, as modelled by BSIs.

13 We identified patients admitted to Royal Free Hospital (RFH) between 01/03/2020 and 01/02/2021, aged
14 >18 years and diagnosed with COVID-19 by RT-PCR detection of SARS-CoV-2 from nasopharyngeal swabs.
15 Tocilizumab use originated from routine clinical care delivery or randomised clinical trials after unblinding.
16 COVID-19 associated BSIs were defined by isolation in blood cultures of any bacteria, excluding coagulase
17 negative staphylococci, between 14 days prior to and 60 days after COVID-19 diagnosis. We excluded patients
18 that developed BSIs prior to receiving tocilizumab. To assess dynamic CRP responses, we included only
19 patients with blood parameter measurements performed at least 3 days prior to the onset of BSIs. Clinical,
20 laboratory and drug data extraction, and statistical analyses were performed as previously described⁵. The
21 study was approved by the Research and Innovation Group at RFH, which stated that as this was a
22 retrospective review of routine clinical data, formal ethics approval was not required.

23 Within the COVID-19 patients that met our inclusion criteria, 107 had received tocilizumab, 17 of whom then
24 developed a BSI during their hospital admission (table 1). A separate cohort of 55 COVID-19 patients
25 developed a BSI but had not received tocilizumab (table 1). Tocilizumab use preceding BSIs was more
26 commonly associated with ICU admission, but the BSI organisms were comparable between the groups (table
27 1). In the first week after tocilizumab administration we observed a rapid fall in CRP (fig 1A), but not for total
28 white cell, neutrophil or lymphocyte counts (fig 1A & fig S1). The CRP reduction following tocilizumab was
29 short lived, with CRP concentrations rising within 21 days of tocilizumab receipt (fig 1A). To exclude
30 confounding by bacterial co-infection, a sensitivity analysis on 90 patients that did not develop a BSI following
31 tocilizumab also showed an early reduction followed by a rebound in CRP (fig S2A). A similar pattern was
32 evident in patients that developed a BSI, although CRP concentrations showed less attenuation and greater
33 heterogeneity within the 21-day period since tocilizumab administration (fig S2B).

34 To test the hypothesis that CRP would rise following a BSI independent of prior tocilizumab administration,
35 we compared CRP responses in 17 patients that had received tocilizumab prior to a BSI with 55 patients who
36 had not received tocilizumab. Strikingly, in both cohorts, BSIs resulted in clear CRP elevations (figs 1B & 1C).
37 We calculated the change in CRP across the time of BSI onset to quantitatively compare this CRP rise. As
38 blood samples were not collected daily in all patients, we derived paired sampling by calculating maximal
39 CRP values 2 or 3 days prior to BSI-detecting blood culture collection and maximal CRP up to 2 days after BSI.
40 This approach revealed an increase in CRP following BSI in 76.5% and 75.0% of patients that had or had not
41 received tocilizumab respectively (fig 1D). Moreover, there was no difference in CRP increase between the
42 groups (median CRP change +88 mg/L vs +76 mg/L respectively, $p = 0.67$ by Mann-Whitney test).

43 As patients developed BSIs at varying times following receipt of tocilizumab, we tested the hypothesis that
44 BSI-induced CRP increment would be proportional to the time interval between tocilizumab administration
45 and BSI onset. However, in the 17 patients that both received tocilizumab and subsequently developed a BSI,
46 no relationship was observed between the length of the tocilizumab-BSI interval and the change in CRP ($r =$
47 0.1069 , $p=0.6811$ by Rank-spearman correlation) (fig 1E).

48 By inhibiting IL-6 signalling, tocilizumab may impact CRP-guided antibiotic prescribing decisions^{5,9}. However,
49 we demonstrate that prior administration of a single dose of tocilizumab does not attenuate CRP responses
50 following a BSI, retaining the utility of this biomarker to diagnose bacterial co-infections associated with
51 COVID-19. These findings have important implications for tocilizumab-treated COVID-19 patients: first,
52 clinically-indicated antibiotic prescriptions are unlikely to be delayed, and second, low CRP levels alone are
53 not an indication for continued prescription of unnecessary antibiotics, supporting stewardship efforts.
54 Nevertheless, BSI onset did not initiate CRP elevations in all patients, irrespective of prior tocilizumab use,
55 emphasising that CRP is only one contributor to diagnosing incipient bacterial infections.

56 Despite preserved CRP responses to BSI, tocilizumab transiently reduced baseline CRP levels, mostly
57 recovering within 21 days. Furthermore, BSI-associated CRP increments were unrelated to time since
58 tocilizumab, indicating that single tocilizumab dosing may not completely neutralise IL-6 responses¹⁰,
59 although a role for IL-6-independent CRP stimuli cannot be excluded. Measuring IL-6 signalling activity in vivo
60 may predict attenuation of CRP responses and inform the need for further tocilizumab dosing in COVID-19⁷.

61 Our study was limited by its single-centre and retrospective nature, constraining patient numbers and
62 negating correction for potential confounders. Nevertheless, increased frequency of corticosteroid use in
63 tocilizumab recipients could have further attenuated CRP responses, counter to our observations. BSIs
64 provided a standardised definition for bacterial infections, but limited extrapolation to non-BSI settings, an
65 area of required future work to confirm the generalisability of our findings.

66 In conclusion, we show that tocilizumab use in severe COVID-19 preserves elevations in CRP concentration
67 following the onset of a confirmed bacterial co-infection, as modelled by BSIs. Use of tocilizumab should not
68 negate judicious, CRP-guided use of antibiotics in COVID-19.

69

70 Transparency declaration

71 Conflict of interests

72 We declare that all authors have no conflicts of interest

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75 Contributions

76 EQW, IB, SB and GP conceived the study. EQW, CB, AN, BOF, JP, ML, SY, SH, DM, MS and GP collected and
77 analysed the data. EQW, SB and GP drafted the manuscript. All authors reviewed and approved the final
78 version of the manuscript.

79 Figure legend

80 **Figure 1. Inflammatory marker responses following tocilizumab administration and onset of BSI in COVID-**
81 **19.** (A) CRP concentration (left panel) and white cell count (right panel) relative to time since tocilizumab
82 administration. All COVID-19 patients receiving tocilizumab included in analysis, independent of a
83 subsequent presence of BSI (n = 107). (B) CRP concentration over time in patients that received tocilizumab
84 and subsequently developed a BSI (n = 17). (C) CRP concentration of time in COVID-19 patients that
85 developed a BSI but with no prior tocilizumab exposure (n = 55). (D) Change (Δ) in CRP induced by onset of
86 BSI in COVID-19 patients stratified by prior receipt of tocilizumab. (E) Relationship between Δ CRP induced by
87 BSI and time between administration of tocilizumab and the onset of BSI. Violin plots represent frequency
88 distribution of all samples, with bold and dashed lines representing median and quartile values. All p values
89 were calculated by Mann-Whitney tests.

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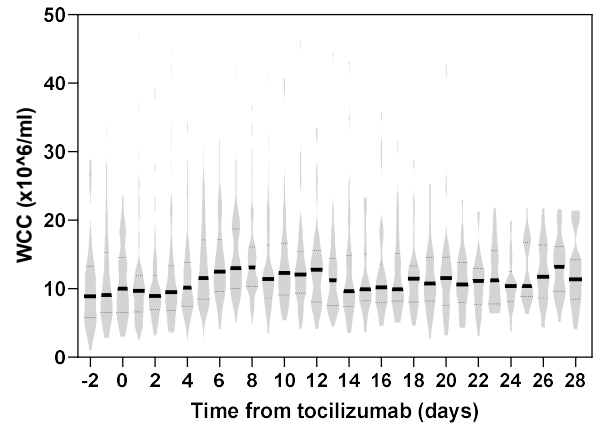
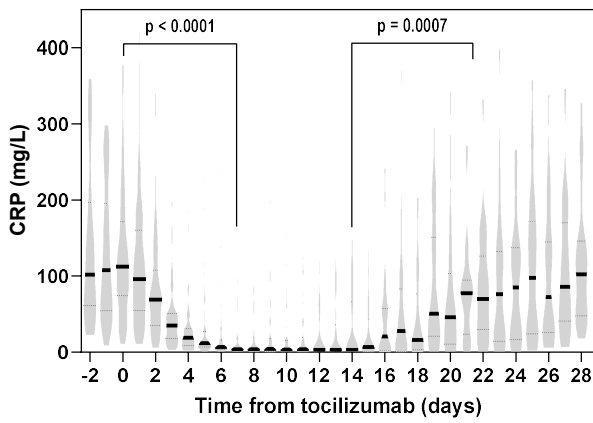
	No BSI & received tocilizumab (n = 90)	BSI & received tocilizumab (n = 17)	BSI & did not receive tocilizumab (n = 55)	p value*
Age , years, median (range)	63 (28-90)	61 (50-78)	63 (31-100)	p = 0.383
Gender , n (%)				p = 0.253
Male	56 (62)	9 (53)	38 (69)	
Female	34 (38)	8 (47)	17 (31)	
Ethnicity , n (%)				p = 0.101
White	33 (42)	4 (31)	27 (55)	
Black	7 (9)	3 (23)	7 (14)	
Asian	15 (19)	4 (31)	7 (14)	
Mixed	2 (3)	1 (8)	0 (0)	
Other	21 (27)	1 (8)	8 (16)	
Charlson co-morbidity score , n (%)				p = 0.095
0	57 (63)	4 (24)	27 (49)	
1	25 (28)	11 (65)	17 (31)	
2	6 (7)	1 (6)	7 (13)	
3+	2 (2)	1 (6)	4 (7)	
ICU admission , n (%)				p = 0.015
Yes	52 (58)	17 (100)	39 (72)	
No	38 (42)	0 (0)	15 (28)	
Corticosteroid use , n (%)				p = 0.076
Yes	79 (89)	15 (88)	35 (65)	
No	10 (11)	2 (12)	19 (35)	
BSI organism , n (%)				p = 0.507
Gram-negative bacilli	N/A	10 (40)	32 (62)	
<i>Enterococcus sp.</i>		2 (8)	12 (23)	
<i>Staphylococcus aureus</i>		4 (16)	7 (13)	
Other		1 (4)	1 (2)	

Table 1. Baseline demographics and clinical characteristics for patients included in the study.

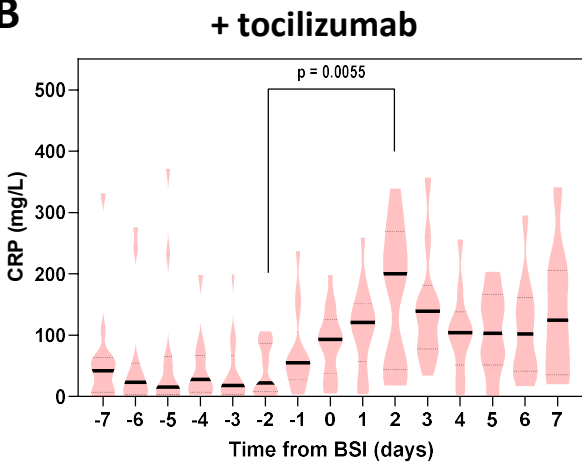
BSI = blood stream infection. * relates to statistical comparison between COVID-19 patients who did or did not receive tocilizumab prior to the onset of BSI. Mann–Whitney test was used to compare age, Fisher’s exact test was used to compare gender and microbiology results, and Chi-square test was used to compare ethnicity and Charlson co-morbidities.

Figure 1

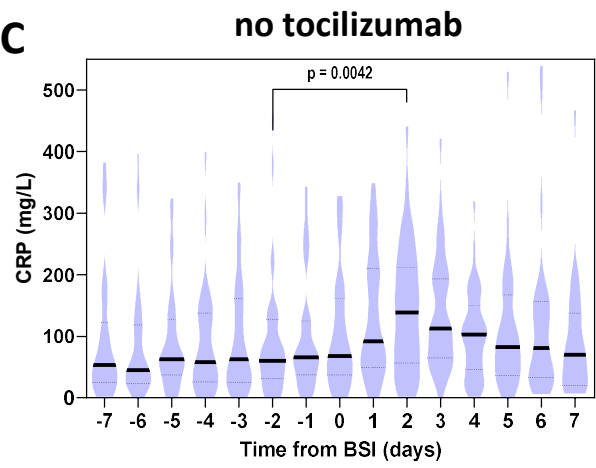
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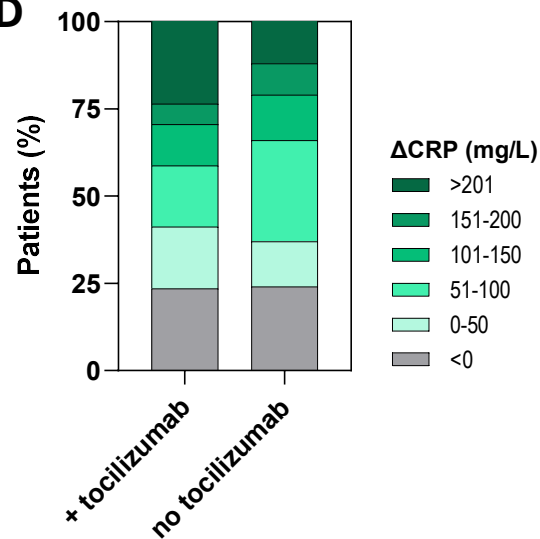
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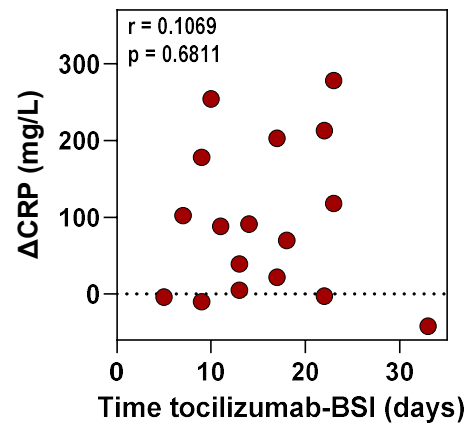
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E



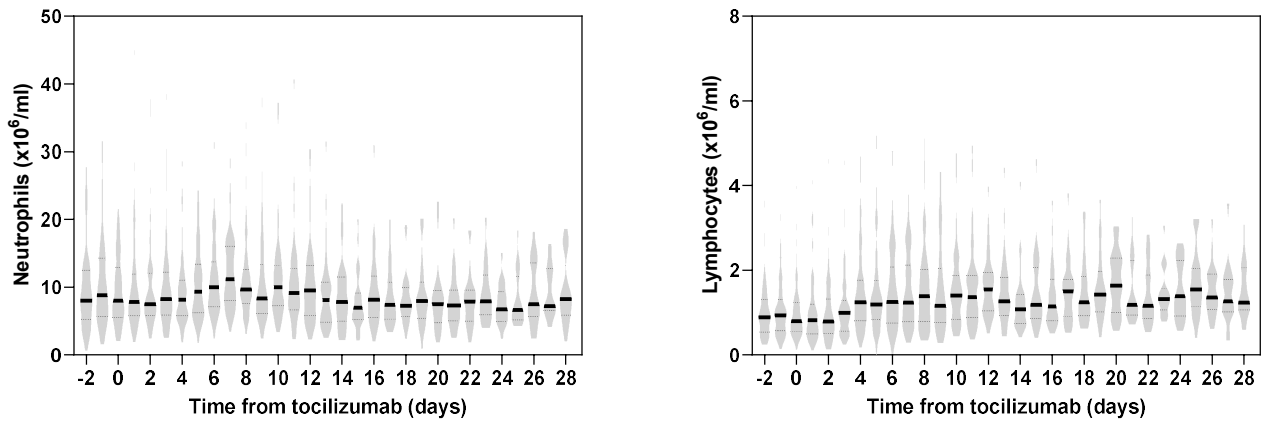


Fig S1. Neutrophil (left panel) and lymphocyte (right panel) counts relative to time since tocilizumab administration. All COVID-19 patients receiving tocilizumab included in analysis, independent of a subsequent presence of BSI (n = 107). Violin plots represent frequency distribution of all samples, with bold and dashed lines representing median and quartile values.

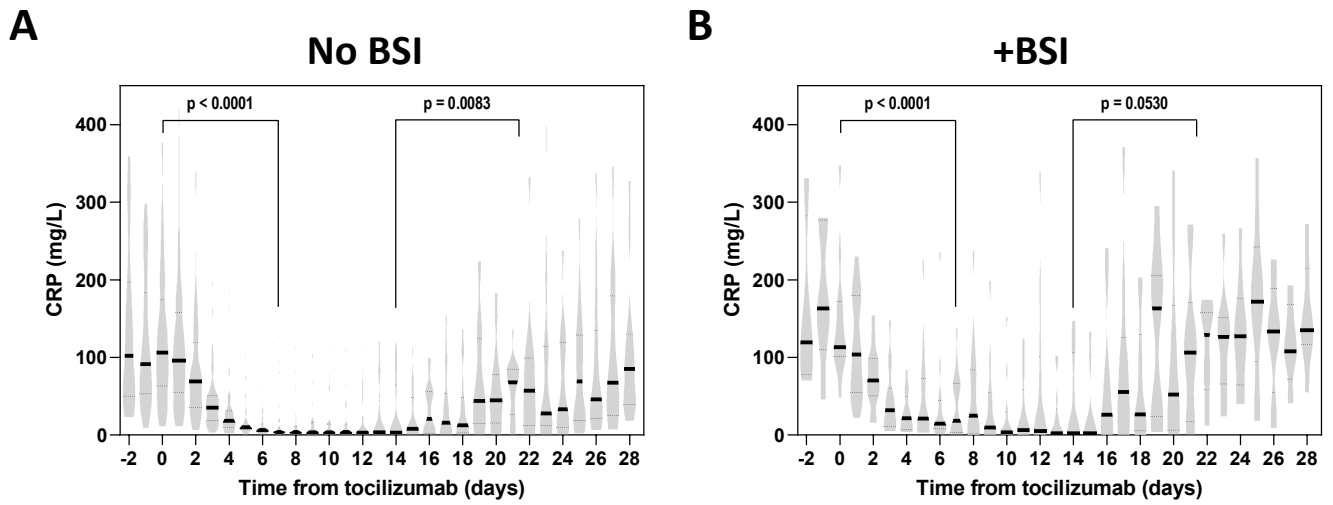


Fig S2. CRP concentration relative to time since tocilizumab administration in patients that (A) did not develop ($n = 90$) or (B) did develop a BSI ($n = 17$) during the same admission. Violin plots represent frequency distribution of all samples, with bold and dashed lines representing median and quartile values.