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To exclude or not to exclude: Considerations and recommendations for C-reactive protein values higher than 10 mg/L

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In recent years, compelling evidence has emerged that chronic activation of inflammatory physiology is implicated in physical and mental health conditions, such as cardiovascular disease, diabetes, depression, and schizophrenia. Since the refinement of more sensitive immunoassays in the 1990s, many studies have used C-reactive protein (CRP) as a reliable and sensitive index of both the acute phase reaction and more sustained inflammatory activation. CRP concentrations typically are below 3 mg/L, but can rise above 500 mg/L during acute illness. When investigating the role of chronic inflammation in human health, it is critically important to differentiate chronic inflammation from an acute inflammatory challenge (e.g., infection/tissue damage) because we typically would expect CRP levels to decrease once an acute challenge resolves. To accurately exclude acutely sick participants, current best practice is to systematically remove all observations when CRP values exceed 10 mg/L (95.2 nmol/L). However, this criterion may inadvertently exclude individuals of interest to studies of human health (Fig. 1).

According to a recent meta-analysis, 42% of 33 studies removed observations with CRP values > 10 mg/L, while 12% applied a more idiosyncratic approach to identify extreme values (e.g., 3 *SD*s above the mean) (Mac Giollabhui et al.). Despite this appearance of consensus, providing a clear justification for this criterion was rare. Designation of 10 mg/L as a cut-off may emanate from a 1981 paper (Shine et al., 1981) that reported that 90% of CRP values fell below the lower limit of detection (3 mg/L) and 99% were < 10 mg/L in 468 volunteer blood donors. It was solely on the basis that the likelihood of observing CRP values > 10 mg/L was small – using outdated assay methods – that the authors concluded: "*values > 10 mg/L are strongly suggestive of an ongoing pathological process*." This cut-off has persisted despite evidence from older (Palosuo et al., 1986) and more recent publications (O'Connor et al., 2009) that CRP values > 10 mg/L are associated with factors other than acute infection. There also is considerable variability of CRP both within and across disease states. In 370 consecutive hospitalized adult patients, median CRP values differed significantly between bacterial infections (120 mg/L), inflammatory diseases (65 mg/L), solid tumor (46 mg/L), non-bacterial infection (32 mg/L), and cardiovascular disease: (6 mg/L). Yet, 33% of patients had CRP values less than 10 mg/L (Keshet et al., 2009). It is now clear that many factors influence circulating CRP values that are unrelated to "pathological" processes (e.g., age, sex, socioeconomic status, race, body mass index, exercise, diet, sleep, medication use) and potential guidelines are available for researchers when deciding to include, exclude, and control for these factors (O'Connor et al., 2009). Thus, it may not always be advisable to routinely exclude participants solely on the basis of one high CRP value. For example, Palosuo et al. (1986) found that whereas 40% of CRP values > 10 mg/L likely were related to acute respiratory infections, 20% seemed to be associated with smoking behavior. Moreover, the distribution of CRP values currently measured in the US population differs profoundly from the 468 volunteer blood donors in the 1981 study. In a nationally representative non-institutionalized

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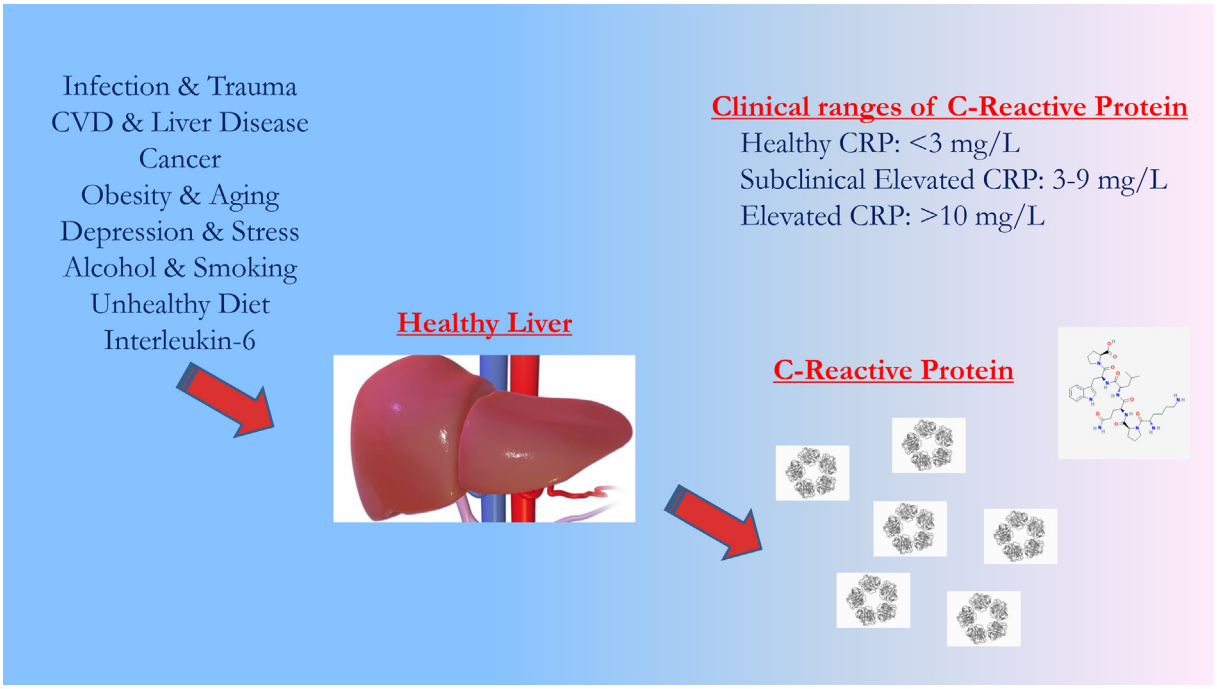

Fig. 1. Potential pathways leading to elevations in C-Reactive Protein.

sample of US adults assessed from 1999 to 2010, 30–40% exhibited CRP levels > 3 mg/L (Ong et al., 2013) compared to the 10% of the distribution referenced above. These differences may be due to sample characteristics, such as inclusion of other racial/ethnic groups in more recent samples who tend to have higher CRP than participants from European backgrounds. Another potential explanation is the dietary and lifestyle changes that have occurred over the last 40 years, which affect CRP levels, such as the steady increase in obesity among both children and adults (Kushner et al., 2006).

Regardless of the specific factors that have resulted in higher levels of CRP, excluding participants from studies may affect the generalizability of findings. Truncated samples will reduce statistical power with unintended ramifications for replication and reproducibility. Could discrepant results arise from strategic decisions about how to handle higher CRP values? Equally important, by excluding CRP values > 10 mg/L, are individuals of potential interest excluded? For instance, the smokers identified by Palosuo et al. (1986) likely would be of central interest to many studies of human health. Heritable factors also can influence circulating levels of CRP (Retterstol et al., 2003), as does assay method, kit manufacturer, and operator skill/experience (Leng et al., 2008). Perhaps of greatest concern, when the factor under study (e.g., depression) correlates with high CRP values, then eliminating individuals with such values may decrease the likelihood of observing an important relationship that actually exists in nature. Thus, it is important to consider whether removing participants with CRP values > 10 mg/L unintentionally excludes individuals of interest to researchers, either because participants engage in more unhealthy behaviors (e.g., smoking, drinking, sedentary), have a heritable propensity for high CRP, or were assessed using a specific methodological approach.

In conclusion, CRP values > 10 mg/L are not always indicative of acute infection/injury. Instead, a more thoughtful approach that recognizes the influence of demographic, behavioral, and technical factors is needed. The question then becomes how best to limit the impact of inherent bias while not inadvertently including sick participants? First, additional steps could be taken to increase confidence that CRP values > 10 mg/L are indicative of an acute immune challenge, either through using a clinical index of the acute phase response/infection, such as interferon gamma-induced protein-10 (Liu et al., 2011), and/or by taking participants' temperature and screening for infection/injury symptoms – it is very likely that this simple screening would identify the suspected cause of very high CRP values (i.e., CRP > 50). Second, one could rule out a competing explanation of elevated CRP by simultaneously evaluating hepatic health with a routine blood biochemistry panel and examining certain liver enzymes (i.e., AST/ALT), which could indicate fatty liver disease. Third, when including extreme values in analyses, researchers may wish to perform a statistical Winsorization, which preserves their rank position in the distribution while lessening skewness. Finally, and potentially most importantly, reporting analyses with extreme values included and then excluded would enable researchers to more thoroughly understand the data.

We hope this commentary will stimulate more discussion and transparency on best practices for employing biomarkers related to inflammation in psychoneuroimmunology research.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2020.01.023.

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