Letter to the Editor

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Clinical laboratory and SARS-CoV-2 infection: where do we stand?

https://doi.org/10.1515/cclm-2020-0372
Received March 24, 2020; accepted March 24, 2020

Keywords: clinical laboratory; immunity; procalcitonin; SARS-CoV-2 infection.

To the Editor,

The current indication for clinical laboratory diagnostics in these early weeks from the upsurge of the SARS-CoV-2 epidemic are essentially targeting the virological aspects, for example, the execution of RT-PCR-based molecular assays. A very exhaustive document indicating the requirements, criteria for a confirmed positivity according to the environment and the patients' conditions and the analytical and preanalytical variables has been made available a few days ago by WHO [1]. In addition, a recently published paper highlights the need to also carefully take into account extra-analytical steps, namely some pre-analytical issues [2]. Besides nucleic acid amplification, this document mentions serology testing, namely assays to detect SARS-CoV-2 specific antibodies, as being one of the next research priorities. While some assays have already become available, several topics are under investigation and discussion on these fields:

1. Dynamic of immunological response
2. Development and validation of useful serological assays
3. Comparative studies of available molecular and serological assays
4. The eventual role of antibodies on protective immunity

During the early course of SARS-CoV-2 infection antibody production is elicited and therefore it has been possible to start generating assays for the detection of IgM and IgG antibodies, targeting the N (nucleocapsid) and/or the S (spike) viral antigens. It has been suggested that IgM-class antibodies may be detected as early as 7 days after the onset of symptoms, followed by IgG after a very short timeframe [3, 4]. Testing for antibodies shall enable to make a diagnosis of ongoing – with a complementary role to viral RNA detection – and past infections. Another key aspect is the potential role of those antibodies in adaptive, or acquired immunity, i.e. immunity towards infection agent after an initial exposure. This usually provides highly specific and long-lived protection against infectious agents and begins with the stimulation of naïve virus-specific T cells that activate and differentiate into effector T cells, mediating the antiviral response and promoting lymphocyte B activation with the eventual surge of antibody response [5]. We do not yet have any clear evidence of protective immunity being raised towards SARS-CoV-2. A first experiment on non-human primates (macaques) hints that animals who survive a first episode do not get re-infected when challenged again with the same agent [6]. However, only two animals have been studies and more data – on the human host – are needed to draw any conclusion.

Out of urgency on focusing on etiologic diagnosis and the paucity of systematic and reliable data, much less attention has been devoted so far to the potential role of other diagnostic assays in the management of patients with COVID-19, i.e. the severe form of viral pneumonia caused by SARS-CoV-2. Some early reports have started to shed some light on this; the following sections will detail with the main findings.

Procalcitonin (PCT)

The frequency of raised (>0.5 μg/L) PCT levels in COVID-19 patients at admittance in a cohort of 1099 Chinese patients has been reported as 5.5% [7]. This is not surprising as the synthesis of this biomarker is inhibited by interferon (INF)-γ, whose concentration increases during viral infections. On the other hand, patients with raised levels at admission bear a significantly higher risk of developing a bacterial infection as PCT production and

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release increases abruptly during bacterial infections. A very recent meta-analysis [8] has shown a cumulative OR of 4.76 (95% CI, 2.74–8.29), with no major differences or inconsistencies among the four studies analyzed, for PCT above the normal reference range for predicting severe COVID-19.

Some papers have also reported on the frequency of septic shock as the cause of death in COVID-19 patients. Results are very conflicting: while Zhou et al. [9] report that 100% of patients who died with COVID-19 had developed sepsis, in the much larger study previously mentioned [7] the frequency of shock during hospital admission was 1.1%, and raised to 13.4% among patients who met a composite endpoint for adverse outcome of ICU admission, need for invasive ventilation or death – the latter occurring in 1.4% of the whole cohort. Furthermore, it shall be considered that those data have been obtained on Chinese patients with a median age at admission between 40 and 50 years and the number of deceased patients considered in not high – 54 and 14 cases, respectively [7, 9]. In the other country who has reported a high mortality so far – Italy – 85.6% of 3200 deceased patients were aged 70 years or older and the median age was 78.7 years (median: 80 years) [10]. Comorbidities have been reported in 98.8% of the 481 patients for which data are available, and this raises a debate on the primary cause of death, i.e. "by" SARS-CoV-19 or "with" SARS-CoV-19. Interestingly, the most frequent complications reported in those 3200 patients were respiratory failure (96.5%), acute kidney injury (AKI, 29.2%), acute myocardial injury (AMI, 10.4%) and finally superinfections (8.5%). This suggests that in this completely different setting biomarkers of AKI, such as NGAL, and/or AMI, such as cardiac troponin, may also be very useful for diagnostic and prognostic purposes.

To conclude on PCT and COVID-19, it appears safe and clinically relevant to indicate that PCT testing upon admission to the intensive care unit should add useful information for early risk assessment and initial rule-out of a bacterial coinfection. PCT monitoring shall allow to identify infections that may occur later and, in case such an event occurs, to monitor progression to the more severe states (sepsis and septic shock).

Other routine parameters

The rates of abnormal values for several hematological and biochemical parameters have been reported in another recent meta-analysis [11]. Much like the PCT data, those observations also have the very relevant bias of having been obtained from patients originating from the same country (China) and possibly with different comorbidities than in other geographical areas. Therefore, it seems sensible to consider those with a more definite pattern, and consider which one(s) may be of help in patient management. Based on that meta-analysis, the three parameters that are most often altered in SARS-CoV-2 infection are lymphopenia, raised C-reactive protein – both common findings in acute viral infections – and D-dimer. This latter has also been studied, along with interleukin-6 (IL-6), in a very recent study comparing adult patients with mild and severe COVID-19 disease [12]. The area under the ROC curve (AUC) of both parameters combined was 0.840, with a sensitivity and specificity for severe disease of tandem testing being 93.3% and 96.4%, respectively.

In conclusion, while the etiological diagnosis of COVID-19 has already been established within a very short time from the recognition of this new clinical entity, progress on the role of known and new diagnostic biomarkers is still needed to provide guidance to clinicians and help laboratory professionals establish the right value and relevance of those on the diagnosis, prognosis and monitoring of SARS-CoV-2 infection.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: Authors state no conflict of interest.

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