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# Complement Activation during Critical Illness: Current Findings and an Outlook in the Era of COVID-19

*Esse quam videri*
—Marcus Tullius Cicero

The complement system is often underestimated. Progress of complement research and consequential clinical applications seem slow but steady. The first glimpse of the existence of complement system was obtained by Jules Bordet (a later Nobel prize laureate) during his pioneering work in the late 19th century. Thereafter, it took over one-hundred years until the first complement inhibitor, eculizumab, received approval by the U.S. Food and Drug Administration in 2007. Eculizumab is a humanized anti-C5 (complement component 5) antibody preventing the cleavage of C5 into C5a and C5b, the central converging point of all pathways of complement activation (Figure 1). Eculizumab improves the survival of patients with paroxysmal nocturnal hemoglobinuria (1). It is also effective for atypical hemolytic uremic syndrome and neuromyelitis optica spectrum disorder (2). These three disease entities have in common the fact that before the introduction of anticomplement therapy, either no choices or only very limited choices of other drugs were available. This recent success story of complement inhibition has refueled a broader interest in this ancient system of innate immune defense for prognostic, diagnostic, and therapeutic exploitation.

In their current work in this issue of the *Journal*, Bain and colleagues (pp. 230–240) report on the association between alternative complement pathway activity and better survival in patients with critical illness (3). The alternative pathway hemolytic assay (AH50) and total complement activity (CH50) tests were retrospectively analyzed in a single-center heterogeneous cohort of $n = 321$ patients with acute respiratory distress syndrome (33%), with suspected sepsis (63%), and on mechanical ventilation (96%). Samples from the first 2 days after ICU admission were measured using commercially available, non–U.S. Food and Drug Administration approved tests. Of note, complement diagnostic tests can be challenging, and sophisticated functional assays have limitations. The patients with a depleted AH50 activity (i.e., below the statistical median of the cohort) had a higher probability of 30-day mortality (36% vs. 22%) and lower 1-year survival. These correlations were not observed for CH50. Preserved AH50 activity correlated with higher serum concentrations of alternative pathway proteins (factor B, factor H, and properdin) and a "hypoinflammatory" phenotype (bicarbonate, IL-8, and TNFR1) but did not correlate with the used definition of immune suppression. Survivors of critical illness showed increased transcriptional expression of complement genes in peripheral blood cells. A higher alternative pathway activity was associated with a lower frequency of bacteremia. Lastly, mice with deficiency of C3 or factor B were prone to splenic dissemination of *Klebsiella pneumoniae* infection.

So, what disease mechanisms could explain the described correlation between higher AH50 and better survival of critical illness? The alternative pathway of the complement system is activated by spontaneous hydrolysis of C3 on foreign surfaces of pathogens (Figure 1), which, unlike host cells, lack the presence of complement inhibitory surface proteins (CD46 and CD55). Complement activation mediates pathogen clearance by the formation of the membrane-attack complex, opsonization for phagocytosis, and modulation of inflammation by chemotactic immune-regulatory anaphylatoxins (Figure 1) (4). Hence, alternative pathway activity may provide control of bacterial infections as a protective mechanism of host defense. Survivors of critical illness may simply have higher capacities of complement protein production or a superior ability to rapidly initiate and de-escalate complement activity, as the authors discuss. Another viewpoint is that low AH50 could denote patients after exuberant complement consumption. Inappropriate complement activity may result in the unloading of harmful effector functions on host cells, with the consequence of disease-causing tissue injury and organ dysfunction during critical illness. The harmfulness of complement overactivation is underscored by the fact that cobra venom factor from poisonous snakes hijacks the alternative pathway, with clearly adverse effects for the host. Therefore, it seems premature to consider whether therapeutic infusions of alternative complement proteins could increase the survival of patients with critical illnesses.



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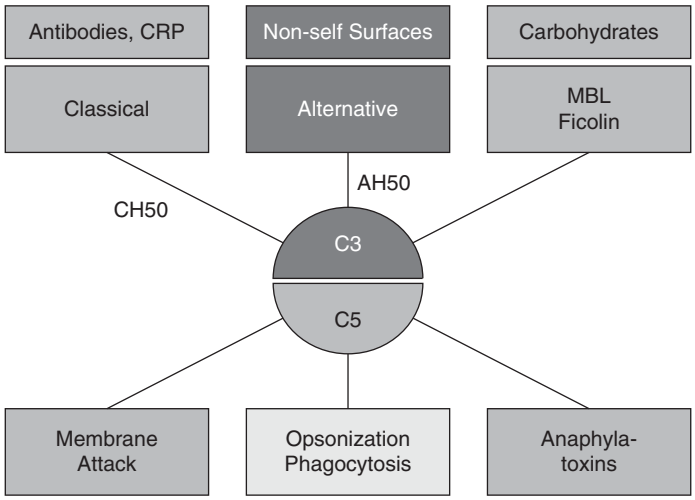


**Figure 1.** Schematic of the complement system. The classical pathway is activated by CRP (C-reactive protein) and IgM and IgG antibodies when bound to antigens (laboratory test: CH50). The alternative pathway is activated by the autolytic cleavage of C3 (complement component 3) on foreign structures (including LPS and zymosan), which are devoid of host complement-inhibitory proteins (laboratory test: AH50). The MBL pathway is activated by pathogen-associated carbohydrates, and the ficolins recognize acetylated saccharides. All pathways converge for amplification at the level of the proteolytic C3/C5 convertases. The effector functions are the formation of the membrane attack complex (C5b–C9), pathogen opsonization for increased phagocytosis, and generation of immune-regulatory anaphylatoxins (C3a and C5a). AH50 = alternative pathway hemolytic assay; CH50 = total complement activity; MBL = mannose-binding lectin.

Although the biorepository of the current study was collected in the era before emergence of coronavirus disease (COVID-19), it is tempting to speculate about potential implications for the current pandemic. In the last few months, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections resulted in a great number of mechanically ventilated ICU patients suffering from sepsis and acute respiratory distress syndrome. Prognostic stratifications and therapeutic interventions are desperately needed.

The complement system is suspected to act in a critical role during the development of COVID-19 (5). Complement activation may occur early during SARS-CoV-2 infection by the direct interaction of viral proteins with the MBL (mannose-binding lectin) and ficolin pathway, rather than the alternative pathway. The viral N (nucleocapsid) protein binds to MASP-2 (mannose-binding protein-associated serine protease 2), a key protease of the MBL pathway leading to overactivation of the complement system and worsening of lung injury (6). In addition, the S (spike) protein of SARS-CoV-2 may possibly directly bind to ficolins. The production of SARS-CoV-2–specific IgM and IgG during approximately the second week of disease may further activate the complement system via the classical pathway, whereas IgA can trigger the MBL pathway (7). The extensive deposition of complement C4d and the terminal complement membrane-attack complex C5b–C9 has been detected in septal capillaries and interalveolar septa of the lungs from COVID-19 autopsies (8). Interestingly, C4d depositions colocalized with MASP-2 and immunohistochemistry for viral S protein (8). A mouse-adapted SARS-CoV strain resulted in complement system activation as early as Day 1 after infection (9). In this model, $C3^{-/-}$ mice displayed significantly less body weight loss and improved respiratory function (9). $C3^{-/-}$ mice showed milder lung pathology and lower cytokine/chemokine concentrations, accompanied by reduced numbers of neutrophils and inflammatory monocytes in the lungs (9). In another experimental study, the host transcriptional response in SARS-CoV-2–infected A549 human lung epithelial cells included the differential expression of C3, C1R, and other complement proteins (10). Serum C3 was reduced in the majority of individuals (57%) from a small cohort ($n = 14$) of healthcare workers infected with SARS-CoV-2, suggesting complement activation and C3 consumption (11). Antibody blockade of the C5a–C5aR1 axis reduced lung injury in a transgenic mouse model of infection with Middle East respiratory syndrome–related coronavirus (12). The inhibition of complement pathways may have beneficial effects on COVID-19–associated coagulopathy (13). Serum C5a concentrations are significantly elevated in patients with severe COVID-19 compared to patients with mild disease or healthy control subjects (6). A phase II monoclonal neutralizing anti-C5a antibody (IFX-1) has been approved for clinical trials in patients with COVID-19 by Chinese authorities and in Europe (14). The compstatin-based C3 inhibitor AMY-101 has recently been safely and successfully used for the first time in a patient with COVID-19–associated pneumonia (15).

Although the current evidence is far from complete, it warrants further studies on the involvement of complement activation in critical illness. The development of better diagnostic tests and prognostic algorithms and the identification of therapeutic targets seems to have upmost importance. It should be cautioned that some complement effects could be context dependent on other host factors. The intricate balance of beneficial and harmful complement functions could substantially differ between specific pathogens. ■

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# Radiomics-based Management of Indeterminate Lung Nodules? Are We There Yet?

With an estimated 229,000 new cases and 136,000 deaths in the United States alone, lung cancer remains the deadliest malignancy worldwide (1). Recently, however, the NLST (National Lung Screening Trial) and the NELSON (Dutch-Belgian Randomized Lung Cancer Screening Trial) studies have demonstrated improved lung cancer mortality for low-dose computed tomographic (CT) screening of the chest in high-risk individuals, and, consequently, lung cancer screening programs are being implemented globally (2, 3). Although this is very exciting, numerous challenges remain, including the detection of large numbers of benign pulmonary nodules, diagnosis of indolent lung cancers, and many others.

The implementation of lung cancer screening and the increased use of diagnostic chest CT, together with advances in CT technology, will undoubtedly lead to an ever-increasing number of detected lung nodules. An estimated 20 million chest CT scans are being performed annually in the United States alone (4, 5).

Despite the reliance on predictive models and nodule-management practice guidelines, considerable variability in nodule classification and uncertainty in management remain (6, 7). Continued research exploring new biological and imaging-based biomarkers is crucial to meeting these challenges.

In this issue of the *Journal*, Massion and colleagues (pp. 241–249) report the development and external validation of a novel, computer-aided, deep learning–based radiomic model, the Lung Cancer Prediction Convolutional Neural Network (LCP-CNN), to distinguish benign nodules from malignant screen-detected and incidentally detected indeterminate pulmonary nodules (8).

Radiomics refers to the identification, extraction, quantification, and analysis of imaging features from radiologic images, with the goal of better or more consistently characterizing radiologic findings. For lung nodules, quantitative and qualitative density and morphologic features provide objective characterization not available by standard visual image interpretation. The analysis of already-available imaging data renders this approach to development and validation of nodule radiomics safe and cost effective. In contrast to conventional radiomic methods in which imaging features are selected by experienced clinicians, deep learning–based radiomics relies on machine learning–extracted features that are frequently abstract and commonly difficult to link back to the underlying biology.

Several other recent studies have explored the potential role of radiomics in the classification of indeterminate pulmonary nodules with promising results (9–11). Enthusiasm has, however, been tempered by the lack of consistency in radiomics features included in these models, the need for homogeneous image acquisition, a lack of stability of the imaging features, the small numbers of scans in relationship to the extracted imaging features (type I error), and a lack of external validation. Models derived from large, heterogeneous real-life data sets, such as the NLST, that are further validated in external data sets, as in the current study, are needed.



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