

# Does COVID-19 Provide a Clue for Thrombosis in ITP?

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One of the significant clinical manifestations of COVID-19 is thrombosis in the different circulatory beds, most commonly within pulmonary vasculature. Pathogenesis of this complication has not yet been elucidated, though some autopsy reports have shown high prevalence of microthrombi in various organs, including lungs, kidney, and heart.<sup>1,2</sup> One of these reports suggested the possibility of circulating megakaryocytes (MKs) as being contributory to this thrombotic feature.<sup>2</sup> The concept of MKs as contributory to thrombosis and a related complication, fibrosis, was recently reviewed.<sup>3</sup> Similarly, MKs may be hypothesized to play a role in the development of thrombosis in patients with immune thrombocytopenia (ITP).

Epidemiological studies were conducted in patients with ITP, and thrombosis was noted in those receiving thrombopoietin receptor agonists (TPO-RA).<sup>4</sup> These revealed an increased risk of thrombosis even in the absence of any ITP-specific treatments. Possible reasons are still being investigated but include some well-known thrombotic risk factors like older age, splenectomy, and antiphospholipid syndrome.<sup>4</sup> One of the culprits may be the circulating MKs similar to that found in COVID-19. This is especially true since there is increased MK activity in both ITP and its treatment with TPO-RA.<sup>5,6</sup>

Beside some forms of leukemias, the concept of circulating MKs in other nonneoplastic conditions is not new with the first observations made over six decades ago.<sup>7</sup> Despite these early findings, the idea that the large platelet precursors being present in the circulation and even producing platelets in the pulmonary vascular bed has been a matter of debate. Even while some experts support a link between lung MKs and cardiorespiratory diseases, many opposing advocates argue that the finding of MKs in lung fields is a gravity phenomenon seen only in the postmortem period.<sup>8</sup> Elegant work from Lefrançois et al has perhaps put an end to the debate and cemented the importance of lung MKs in platelet production, and it can confidently be said that at least a part of platelet pool can be generated within the lungs.<sup>9</sup> In addition to the lungs, the placenta has also been shown to

be a site of platelet production.<sup>10</sup> The lungs being “nonfunctional” in the fetus, perfusion studies of placentae were undertaken by investigators who were able to isolate MKs with copious cytoplasm from this fetal respiratory organ.<sup>10</sup> This study gives additional proof to the concept of circulating MKs and platelet production in sites other than the bone marrow, including the lungs. In this context, we have to wonder what may be the role of these circulating MKs.

Thrombocytosis is a common accompaniment of inflammatory states. It is also known to the platelet biologists that inflammatory cytokines like interleukins can stimulate MKs to produce platelets. This is indeed the case as has been demonstrated in patients with lung inflammation.<sup>11</sup> Hansen and Pedersen collected blood from cubital veins and analyzed for MKs in 30 patients with bronchitis or bronchopneumonia and identified significantly higher circulating MKs in these patients.<sup>11</sup> It is possible that the circulating MKs participate in the host-defense process by releasing chemokines and producing many young, active platelets at the inflammatory site (e.g., the lungs). Since platelets do not have the synthetic machinery to respond to the various infectious or inflammatory stimuli in a rapid manner (despite recent evidence of platelet mRNA<sup>12</sup>), delivery of a large number of well-equipped platelets in the vicinity of infection or inflammation is far more advantageous than a distant release of platelets.

## But Do MKs Contribute to Thrombosis?

Autopsy reports of patients who succumbed to COVID-19 provide proof to this possibility, along with the demonstration of several naked MK nuclei in the lung tissue.<sup>13</sup> An Italian study reported on the autopsy data from nearly 40 patients and identified an increased number of CD61 antibody-positive MKs in the lung capillaries of almost all patients.<sup>14</sup> They however did not correlate the MK load with the microthrombi which were omnipresent in these lung samples. The MK–thrombosis link was, however, shown in an older publication,

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wherein a significant correlation has been identified between MK number and fibrin microthrombi in lung biopsies of 22 patients who died from extensive burns.<sup>15</sup> Returning to COVID-19, Rapkiewicz and colleagues found MKs in the microvasculature of the heart, renal glomeruli, and lungs. The important finding in this study was the colocalization of MKs with platelet–fibrin microthrombi in the pulmonary circulation and even in the heart microvasculature.<sup>2</sup>

It has recently been suggested that TPO can trigger MK-biased hematopoietic stem cells to express von Willebrand factor (VWF), which is upregulated during times of stress such as inflammation.<sup>16</sup> MKs also contain ultralarge high-molecular-weight VWF multimers not present in plasma, which may be released in the inflammatory setting.<sup>17</sup> Elevated VWF levels are a well-established risk factor for thrombosis and have been postulated as one mechanism for COVID-19 thrombosis.<sup>18,19</sup> In relation to ITP, VWF levels were noted to be elevated especially in older patients (as mentioned before, a risk factor for thrombosis) in a study of 20 patients with platelet count less than  $100 \times 10^9/L$ .<sup>20</sup> This rebalanced hemostasis (high VWF levels in those with lower platelet counts) would explain the reduced bleeding tendency commonly observed in ITP patients despite the very low platelet counts, in comparison to hypoproliferative thrombocytopenia where there are fewer MKs and therefore possibly no increased VWF release. This balance may however be tipped toward thrombosis in patients with ITP when they acquire additional risk factors (e.g., advanced age, splenectomy, inflammatory diseases which cause secondary ITP). In addition, TPO-RA treatment could also influence the MK–TPO axis by increasing VWF levels and predispose some of these patients to develop thrombosis. Almost three decades ago, a significant increase in VWF ristocetin cofactor levels was identified in patients with ITP, but no relationship to thrombosis was described.<sup>21</sup> More recently, a study published in abstract form described elevated plasma VWF levels in approximately 50 patients with ITP while the ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) levels were lower in the same patients compared with control subjects.<sup>22</sup> An increased ratio of VWF:ADAMTS13 was suggested by these authors as possibly contributory to the pathogenesis of thrombosis in ITP.<sup>22</sup> Such a relative ADAMTS-13 deficiency was also demonstrated in a cohort of 50 hospitalized COVID-19 patients. The study demonstrated an inverse correlation between D-dimer and ADAMTS13 suggesting high possibility of thrombotic microangiopathy, where the VWF–platelet axis plays a pathogenic role.<sup>23</sup>

In conclusion, circulating MKs may play a role in triggering thrombosis in patients with conditions like COVID-19 and ITP (and its treatment with TPO-RA). This is possibly linked to release of VWF by MKs, which are increased in number in response to increased TPO levels in these disease states. Future, large studies in this interesting area are warranted.

#### Conflict of Interest

J. T. reports personal fees from AMGEN, personal fees from NOVARTIS, outside the submitted work.

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