An extensive database analysis demonstrates significant increase in platelet quantity in unselected hospitalized patients following treatment with oseltamivir

Thrombocytopenia is a known risk factor for morbidity in individuals with various health conditions, including malignancy. Thrombocytopenia can lead to adverse outcomes including increased bleeding risk and mortality.[1-3] Preventing and correcting thrombocytopenia may mitigate these consequences and improve patients' outcomes.[3] One proposed mechanism for remedying thrombocytopenia is to prolong the lifespan of platelets by interfering with normal platelet clearance. In addition to apoptosis, platelets are marked as aged and removed from the circulation by hepatocytes and hepatic Küpffer cells in a highly coordinated process triggered by the loss of the terminal carbohydrate moiety sialic acid (platelet desialylation).[4]

Neuraminidases cleave the glycosidic link and remove the terminal sialic acid. This allows the platelet to bind to the hepatic Ashwell-Morell receptor[5] (likely also the macrophage galactose lectin on Küpffer cells) and be removed from the circulation.[6] Thus, oseltamivir, a known neuraminidase inhibitor commonly used in the treatment of influenza, may have off-target effects by halting platelet desialylation and attenuating platelet apoptosis and phagocytosis.[7] Based on this mechanistic observation, there is clinical interest in applying this finding to treat immune thrombocytopenia (ITP). Previous *in vitro* and *in vivo* studies have specifically demonstrated the important role of desialylation in the clearance of platelets in ITP, and its possibilities as a therapeutic target in ITP.[8-11]

Previously, only a small pilot study[11] had demonstrated an increase in platelets in response to oseltamivir in patients with ITP until a recent multicenter, randomized phase II study. In this study, individuals with ITP were randomly assigned to either dexamethasone monotherapy or dexamethasone in combination with a 4-day course of oseltamivir. Researchers randomized 96 patients and reported that the group given the combination treatment achieved significantly higher response rates at both 14 days and 6 months.[12]

We were interested in whether we could confirm that treatment with oseltamivir is independently associated with a significant change in platelet counts before and after treatment. Using a large database, we hypothesized that receiving oseltamivir would increase platelet levels.

We obtained approval for a review of electronic medical records from the Medical College of Wisconsin's Institutional Review Board. Using TriNetX software, we extracted data from patients' charts. In our analysis, we included patients treated in the Medical College of Wisconsin system if they: (i) had at least one clinical encounter between January 1, 2010 and December 31, 2020; (ii) were over 18 years old; (iii) were administered oseltamivir; and (iv) had their platelet count measured at least once a maximum of 30 days before receiving oseltamivir and a post-treatment measurement a maximum of 30 days following the administration of oseltamivir. The platelet counts before and after oseltamivir administration were recorded.

We interrogated patients' charts for demographic data and variables that could affect platelet levels, including a positive influenza polymerase chain reaction test within 10 days of oseltamivir administration. We utilized codes of the tenth revision of the International Classification of Diseases (ICD10) to identify the variables of interest.

We employed a multivariable nested random effects model to account for the variance of the repeated measurements within the same treatment episode and multiple episodes within the same patient. We performed analyses using SAS 9.4 (SAS Institute, Cary, NC, USA).

We identified 2,168 patients who met the enrollment criteria. Table 1 includes their demographic information as well as comorbid conditions. Some patients had multiple treatment episodes over the 10 years which were at least

Table 1. Demographics of the study population.

	Number (%)
Whole cohort	2,168 (100)
Age group	
18-30 years	148 (6.8)
30-50 years	399 (18.4)
50-65 years	638 (29.4)
65+ years	983 (45.4)
Sex	
Male	965 (44.5)
Female	1,203 (55.5)
Race	
Hispanic	61 (2.8)
Non-Hispanic	2,106 (97.1)
Unknown	1 (0.1)
Positive influenza test	
Yes	656 (27.37)
Comorbid conditions	
Cancer diagnosis	362 (15.10)
Liver disease	321 (13.39)
Alcohol abuse	857 (35.75)
Heparin use	153 (6.38)
Sulfonamide use	100 (4.17)
Glycoprotein use	169 (7.05)

90 days apart, creating 2,397 patient-treatment episodes. We performed *t*-test analysis on the log-transformed fold change of the platelet level. On average there was a 1.14-fold increase in platelet levels after therapy with oseltamivir, with an average increase of 14%. We then broke the population down into four quartiles using baseline platelet levels. The first quartile was composed of 603 patients with a baseline platelet level equal to or less than $153x10^3/\mu L$. The mean fold change for those individuals was 1.40, meaning that platelet level increased, on average, 40% after oseltamivir treatment. Compared with all patients we analyzed, the patients in the lowest quartile were found to have the highest fold change in platelets after oseltamivir administration (Figure 1).

In the current circumstances of scarce blood products, novel mechanisms to prevent or ameliorate severe thrombocytopenia are critical. Similar to other studies,[13,14] our results show an increase in platelet counts after oseltamivir administration, particularly in patients with the lowest baseline platelet count.

One possible explanation could be that oseltamivir increases platelets by a set amount regardless of the initial platelet count, and that the apparently more significant elevations in patients with more severe thrombocytopenia could be explained by starting with a lower platelet count from which to calculate log-fold and percentage increase.

Another possibility could be differing levels of platelet desialylation between the quartiles contributing to different baseline platelet levels, as shown by research demonstrating an inverse correlation between platelet count and the extent of specifically *O*-glycan desialylation in murine models.[15] For example, the patients in the first quartile could have higher levels of platelet desialylation compared to those in the other quartiles, explaining both that quartile's lower platelet baseline and its superior response to the sialidase inhibitor oseltamivir, as there are more therapeutic targets available in this group. A recent prospective cohort study showed that higher levels of desialylation can be found in certain diseases including connective tissue disease, aplastic anemia, and myelodysplastic syndromes.[9] As our search did not specify these precise co-morbid conditions, it is possible that the first quartile contained more patients with these "high-desialylation" diseases, which could be responsible for the lower baseline platelet levels. More research identifying the specific mechanisms of oseltamivir's activity at differing platelet levels would be useful.

Limitations of our work include those inherent to the retrospective cohort design, such as the inability to determine whether the effect of oseltamivir is secondary to the anti-influenza effect or to the glycan effect on platelets. Notably, however, only 27% of the cases in our database had a positive influenza test. Those patients testing positive did not have a significantly different increase in platelets compared to the overall population. This finding supports the hypothesis that the increase in platelets is not associated with recovery from the respiratory illness.

Ultimately, the large sample size in our observational study adds power to the premise that the neuraminidase inhibitor oseltamivir could benefit individuals with severe thrombo-

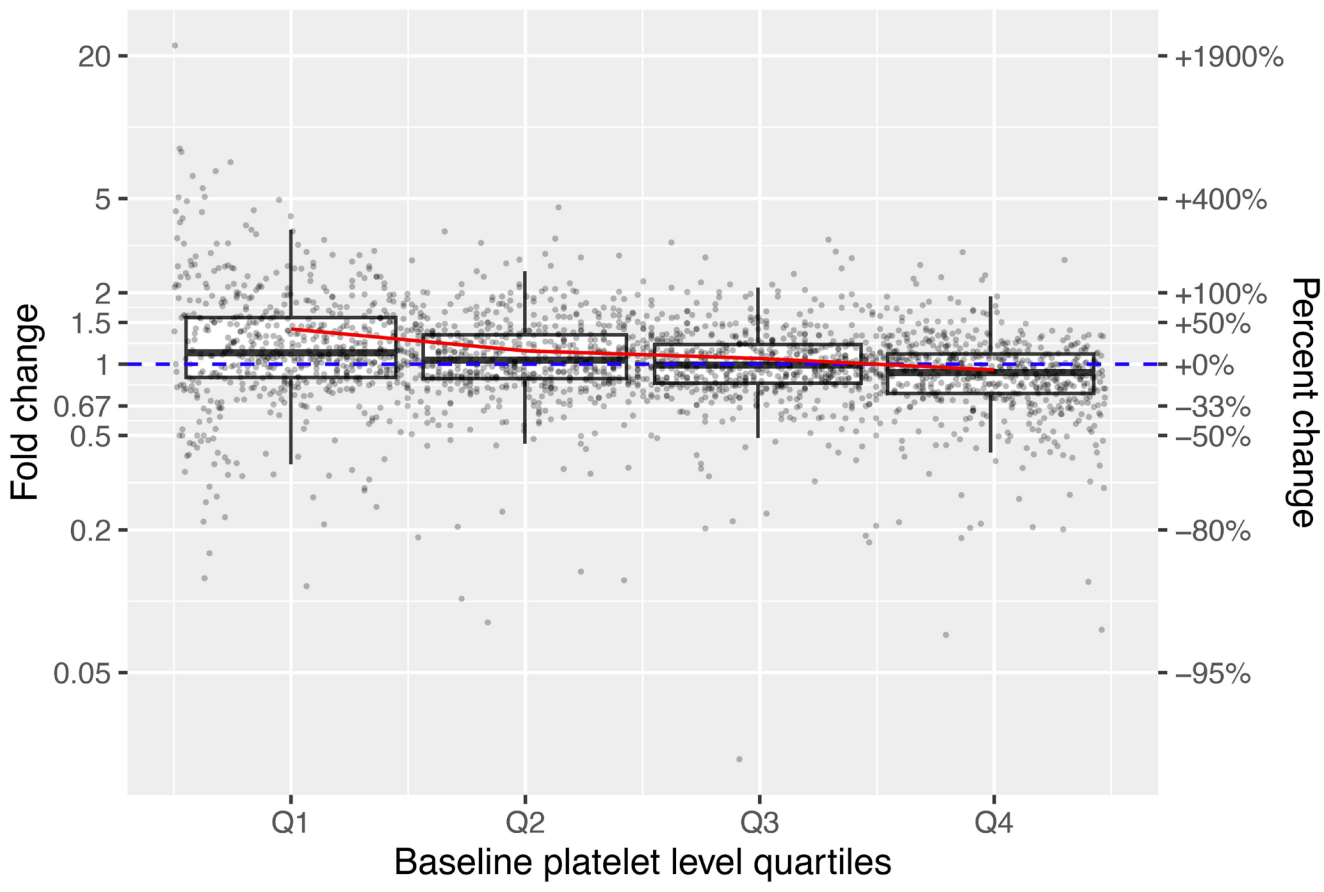

Figure 1. Fold change (left axis) and percent change (right axis) of platelet counts following oseltamivir therapy. Each point represents one subject, ordered from lowest to highest baseline platelet count along the x-axis. The box-and-whisker plots summarize the median and interquartile range from each quartile of baseline platelet count. The dashed blue line represents no change (fold-change = 1, percent-change = 0%). The solid red line connects the means within each quartile of baseline platelet count.

cytopenia even in situations other than immune-mediated thrombocytopenia. We believe that these data provide support for clinical trials to test such a hypothesis prospectively.

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Disclosures

No conflicts of interest to disclose.

Contributions

CM wrote the manuscript and performed research. QL and AS performed statistics and analyzed data. SB collected data and wrote the manuscript. LM, JPB, and KH supervised the study and edited the manuscript.

Data-sharing statement

The data that support the findings of this study are available from the corresponding author, CM, upon reasonable request.

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