Conclusion
The study describes in detail that platelets exist in an active state. Our work clearly demonstrates that elevated levels of activation markers exist on platelet surfaces (P-selectin and PS-exposure, and PAC-1 binding) during early days of infection (day 4 and day 6) which is directly correlated with high genome copies of DV in platelets pellet and inversely correlated with low platelet counts during early days of infection (such as day 4 and day 6) in Dengue-infected patients. The number of platelets is correlated with less activation of platelets as well as non-detectable virus genome in platelets on day 10 of infection. Low platelet numbers on day 4 and day 8 and recovery on day 10 of infection clearly indicate the fine association between elevated platelet activation and decreased platelet count in Dengue patients.

It is well reported that complement protein (C3) and Immunoglobulin (IgG) can bind to the activated platelets surface and promote lysis and clearance of platelets from circulation and upon lysis platelet release microparticles in extracellular milieu which is used as biomarker of platelet activation and lysis. We estimated the levels of bound C3 or IgG on platelets collected from peripheral blood of patients on different days of infection. Increased levels of C3 and IgG on platelet surface correlated with elevated platelet activation but low platelet count on days 3-4 of fever and the decreased levels of C3 and IgG correlated with low platelet activation but normal platelet count on day 10 of infection in Dengue patients.

*In vitro*, after treatment with DV2, platelets (isolated from healthy individuals) showed elevated activation and apoptosis, which was further diminished by the use of platelet activation inhibitor prostacyclin (PGI₂). DV2 treatment to platelets in presence of plasma increased the binding of C3 and IgG on platelet surface in a MOI-dependent manner *in vitro*. DV2 treatment increased platelet lysis and generated more platelet-derived microparticles *in vitro*. DV2 treatment to whole blood increased the
adhesion of platelet and clot formation on endothelium monolayer in vitro. DV2 treatment to platelets increased the phagocytosis of platelets by monocytes (isolated from same healthy individuals) in vitro. The above mechanisms thus suggest the underlying mechanism of platelet clearance from circulation in patients with Dengue infection.

Viruses use host cells for replication and propagation. Although studies explain the mechanisms, but it is not clear yet, how the viral infections are rapidly elevated within 2-3 days after gaining entrance in the host. We have investigated the mechanism using closely related flaviviruses such as Dengue virus (DV).

We describe that the host protein platelet factor 4 (PF4) promoted replication and propagation of DV in monocytes. In presence of either the supernatant of activated-platelets or rhPF4, the synthesis of new virions of DV was increased significantly in monocytes in vitro.

Besides, PF4 decreased the IFN-α secretion in monocytes by inhibiting the phosphorylation of p38MAPK and in turn decreases activity of STAT-2 and IRF-9. Our data from Dengue patients also confirmed direct correlation between PF4 and viral replication. Further the blocking of PF4 receptor CXCR3 with AMG487 treatment significantly decreased DV infection.

Therefore, our study suggests that PF4-CXCR3-IFN axis is a potential target for developing treatment against viral infections including DV. Antagonists to CXCR3 including AMG487 can be useful for treating DV and may be for other viruses.