

1 *Study population*

2 The description of screening and enrolment procedure for pediatric dengue
3 patients enrolled into this study has already been described in previous reports (Kar et
4 al., 2017, Singla et al., 2016). Briefly, children aged between 4-14 years presenting to
5 AIIMS with symptoms suggestive of dengue were screened using a rapid dengue NS1
6 Antigen and differential detection of IgM and IgG antibodies to DENV in serum/plasma
7 (J. Mitra and Co. Pvt. Ltd, New Delhi). Patients testing positive for either NS1 or IgM
8 were informed and requested for consent for the study. We classified all patients at
9 baseline bleeds based on the latest WHO grading into 3 groups — Severe Dengue
10 (SD), Dengue with Warning signs (DW) and Dengue Illness (DI) (2009). Patients were
11 classified as primary and secondary infection based on IgG and IgM ELISA as
12 described before (Singla et al., 2016). DENV serotyping was performed using total RNA
13 isolated from whole blood as described before (Singla et al., 2016).

14

15 *Viral RNA isolation from whole blood and plasma*

16 *RNA from whole blood (250 µl) was isolated using TRIzol-LS reagent (Invitrogen)*
17 *according to manufacturer's instructions. RNA pellet was air dried and resuspended in*
18 *30 µl nuclease-free water.*

19 Total RNA was extracted from plasma samples using QIAamp Viral RNA Mini Kit.
20 140 µl plasma sample was centrifuged at 16,000 x g for 5 min in a refrigerated table top
21 centrifuge to remove any precipitate and processed as per the manufacturer's protocol.
22 Viral RNA was eluted in 60 µl nuclease free water and used for estimation of DENV
23 genome copy numbers as described previously (Agrawal et al., 2013).

24

25 *References*

26

27 Agrawal T, Schu P, Medigeshi GR. Adaptor protein complexes-1 and 3 are involved at
28 distinct stages of flavivirus life-cycle. Sci Rep 2013;3:1813.
29 Kar M, Singla M, Chandele A, Kabra SK, Lodha R, Medigeshi GR. Dengue Virus Entry and
30 Replication Does Not Lead to Productive Infection in Platelets. Open Forum Infect Dis
31 2017;4(2):ofx051.
32 Organization WH. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control:
33 New Edition. WHO Guidelines Approved by the Guidelines Review Committee. Geneva;
34 2009.
35 Singla M, Kar M, Sethi T, Kabra SK, Lodha R, Chandele A, et al. Immune Response to Dengue
36 Virus Infection in Pediatric Patients in New Delhi, India--Association of Viremia,

37 Inflammatory Mediators and Monocytes with Disease Severity. PLoS Negl Trop Dis
38 2016;10(3):e0004497.
39