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# PERSISTENCE OF VIRUS REPLICATION MARKERS IN RESPIRATORY SAMPLES FROM AN EBOV-INFECTED PATIENT

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## Background

From December 2013 to December 2015, the Ebola virus (EBOV) Makona variant has been the causative agent of an unprecedented EBOV outbreak. In Italy, two imported cases have been successfully treated at the National Institute for Infectious Diseases (INMI) "L. Spallanzani", IRCCS, of Rome, Italy. In order to better understand EBOV infection patterns in different body districts, it is mandatory to develop new countermeasures, as well as to fully comprehend the pathways of inter-human transmission. The first Italian imported case admitted for Ebola Virus Disease (EVD), EVD case INMI1, reported in detail elsewhere [1], presented with severe interstitial lung involvement and showed high EBOV viral load in the respiratory tract, which led to the hypothesis that EBOV may replicate into the lung. The present study was aimed at further investigating the contribution of the respiratory tract in viral replication and shedding, using the levels of viral RNAs (vRNA) and cRNA/mRNAs, whose role as indicator of viral replication has been assessed before [2,3]. To this aim, we investigated for the first time the presence and extent of EBOV vRNA and cRNA/mRNAs in a series of clinical specimens (plasma, sputum, nasopharyngeal swab, urine and ocular swab) collected during the first 15 days of hospital stay of the second Italian Ebola patient (EVD case INMI2) who was admitted at INMI with low $O_2$ saturation and basal bilateral interstitial lung involvement at chest X ray.

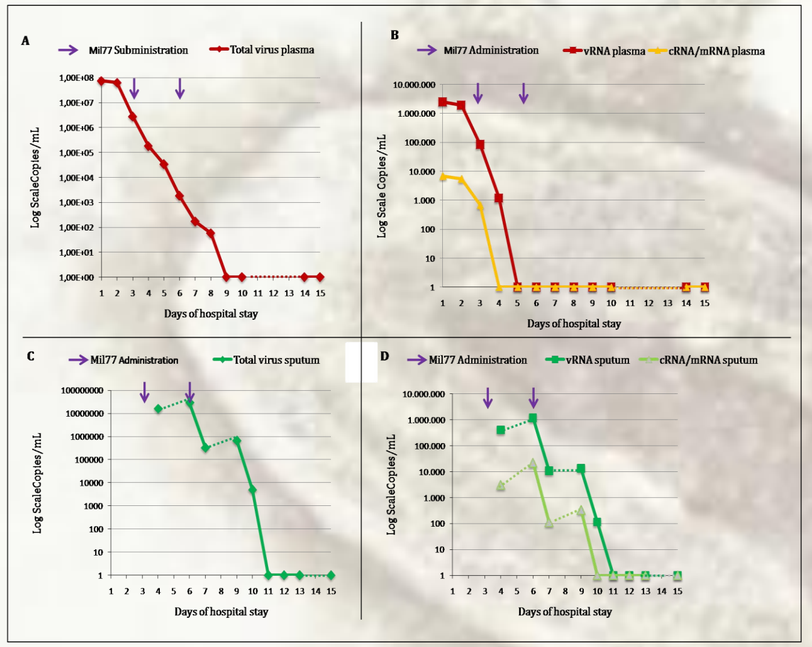


**Figure 1**. **EBOV RNA trends in plasma and sputum specimens during the first 15 days of hospitalization.** Arrows indicate the administration of the experimental drug Mil77 (day 3 and day 6). Dotted lines represent the hypothetical trend of those samples not available for this study. Symbols are specified in the panels.

## Results

**Figure 1** shows EBOV RNA trends in plasma (Panel A) and sputum (Panel C) of the EVD case INMI2. High EBOV viremia has been reported since the first days of hospital stay with a pronounced decrease after the administration of Mil77 (day 3 and 6); and reached undetectable levels on day 9. Differently from plasma, EBOV RNA in sputum remained high up to day 9, with a peak at day 4 and 6, and became undetectable at day 11. High levels of vRNA and cRNA/mRNAs were observed in plasma (Panel B) up to day 5 and day 4 of hospital stay, respectively. In the respiratory compartment, vRNA was present up to day 11, and cRNA/mRNA was present up to day 10 (Panel D). The levels of vRNA and cRNA/mRNA in sputum samples were 2- and 3-fold higher than vRNA and cRNA/mRNA values in plasma, respectively.

The presence of total, vRNA and cRNA/mRNA in nasopharyngeal swabs is shown in **Figure 2**. Total RNA was present at high levels until day 5, was detected at low levels until day 12 and became undetectable at day 13 (Panel A). The long persistence seen in the total RNA was paralleled by the vRNA, which was detectable until day 10 (Panel B), although the cRNA/mRNAs levels became promptly undetectable on day 6 (Panel B). In urine and ocular swab, total RNA levels, as well as vRNA levels, remained detectable up to day 15 and day 4, respectively. Interestingly, cRNA/mRNA levels were always undetectable in all specimens analyzed from these compartments (data not shown).

**Methods:** Inactivation of samples was performed in the BSL4 facility and total RNA extraction in the BSL2 laboratory using QIAamp® Viral RNA Mini Kit (Qiagen). cDNA specific for positive (cRNA/mRNA) and negative (vRNA) strands was obtained by reverse transcription, using TaqMan Reverse Transcription Reagent kit (Applied Biosystems, Foster City, CA, USA), using either reverse or forward primers, respectively, targeting L gene. cDNAs were then amplified using a modified qRT-PCR, RealStar® Filovirus Screen RT-PCR Kit 1.0 (Altona Diagnostics GmbH) which omitted the reverse transcription step.

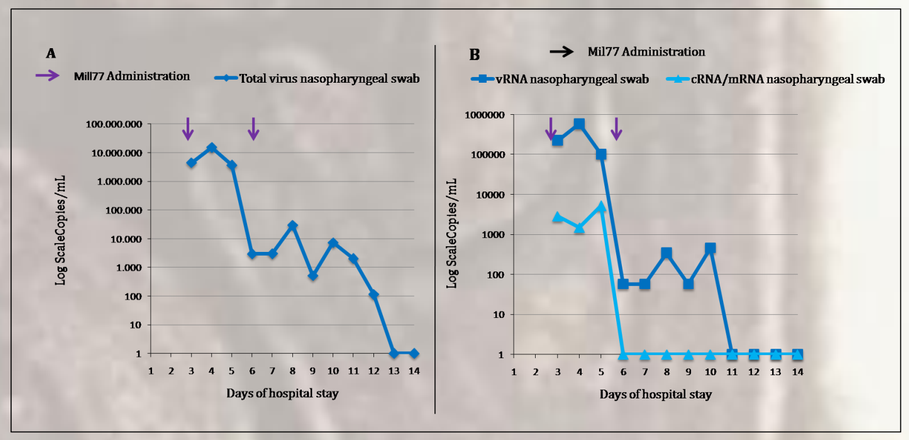


**Figure 2**. **EBOV RNA trends in nasopharyngeal swab specimens during the first 15 days of hospitalization.** Clinical specimens from EVD case INMI2 were collected daily. Arrows indicate the administration of the experimental drug Mil77 (day 3 and day 6).

## Discussion

The detection of replication markers, such as cRNA/mRNA and vRNA, in sputum, up to days 9-10, in front of negative data in plasma since days 4-5, strongly supports ongoing local viral replication in the respiratory tract, rather than plasma spill over or of prolonged RNA stability. Moreover, the trend of total RNA and vRNA in nasopharyngeal swabs was detectable up to day 12 and day 11, respectively, despite the lack of cRNA/mRNA replication markers in the nasopharyngeal swabs from day 6 onward. Since viral clearance from plasma (both for total viral RNA and replication markers) was already achieved at day 9, the residual total RNA and vRNA present in the nasopharyngeal swabs at days 10 and 11 is likely to derive from spill over of active replicating virus present in the lower respiratory tract. Finally, the lack of cRNA/mRNA, in presence of detectable total RNA and vRNA, in both urine and ocular compartments does not suggest ongoing viral replication in these districts. Further investigation is needed in order to better understand the clinical meaning of the respiratory tract in viral replication and shedding, and possible contribution to respiratory impairment.

**Reeferences**

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