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van Griensven, J; Edwards, T; Baize, S; Ebola-Tx Consortium; , COLLABORATORS; van Griensven; Edwards; de Lamballerie, X; Semple, MG; Gallian, P; +20 more... Baize, S; Horby, PW; Raoul, HV; N'Faly, M; Antierens, A; Lomas, C; Faye, O; Sall, AA; Fransen, K; Buyze, J; Ravinetto, R; Tiberghien, P; Claeys, Y; De Crop, M; Lynen, L; Bah, EI; Smith, PG; Delamou, A; De Wegheleire, A; Haba, N; (2016) Efficacy of Convalescent Plasma in Relation to Dose of Ebola Virus Antibodies. *The New England journal of medicine*, 375 (23). pp. 2307-2309. ISSN 0028-4793 DOI: <https://doi.org/10.1056/NEJMc1609116>

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lyze germline-derived data from 402 patients with differentiated thyroid carcinoma. We used these data, which were made available by the Cancer Genome Atlas (TCGA) Research Network,<sup>2</sup> to assess the contribution of known cancer-predisposition genes<sup>3</sup> to differentiated thyroid carcinoma.

No mutations were detected in *PTEN*, *APC*, or *WRN*. Instead, to our surprise, we identified 23 patients (5.7%) with a truncating mutation in one of five DNA-repair genes: *BRCA1* (in 1 patient), *ATM* (in 1), *CHEK2* (in 4), *MSH6* (in 4), and *BRCA2* (in 13). These findings suggest a role for DNA-repair genes in susceptibility to differentiated thyroid carcinoma, but additional deleterious somatic mutations in these genes were not seen in the associated tumors. Studies have identified an excess of differentiated thyroid carcinoma in *CHEK2* mutation carriers,<sup>4</sup> but not in *BRCA2* heterozygotes.<sup>5</sup> Further investigations to clarify the clinical significance of these findings are warranted.

Somayyeh Fahiminiya, Ph.D.

Research Institute of McGill University Health Centre  
Montreal, QC, Canada

Leanne de Kock, B.Tech.

William D. Foulkes, M.B., B.S., Ph.D.

McGill University  
Montreal, QC, Canada  
william.foulkes@mcgill.ca

No potential conflict of interest relevant to this letter was reported.

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DOI: 10.1056/NEJM1613118

**THE AUTHORS REPLY:** In reply to Fahiminiya and associates: epidemiologic studies show that some papillary thyroid carcinomas are heritable, and genomewide association studies have identified

variants conferring predisposition to papillary thyroid carcinoma, although their functional consequences have not been fully elucidated.<sup>1-3</sup> In response to our article, in which we described germline events predisposing to papillary thyroid carcinoma, Fahiminiya and colleagues analyzed germline data from a TCGA study involving patients with papillary thyroid carcinoma, and they identified truncation mutations in DNA-repair genes in 23 of 402 patients (5.7%). This potentially important observation has not yet been confirmed.

Somatic mutations of DNA damage–response genes, including *CHEK2* and *PPM1D*, were detected in high-risk papillary thyroid carcinoma tumors from the series in TCGA. Indeed, mutations of this class of genes are also enriched in poorly differentiated and anaplastic thyroid carcinomas.<sup>4</sup> Although current evidence points to a multifactorial genetic predisposition to papillary thyroid carcinoma, these findings may warrant a reappraisal of the role of DNA-repair genes in susceptibility to papillary thyroid carcinoma, particularly in vulnerable populations such as children who have been exposed to ionizing radiation.

James A. Fagin, M.D.

Memorial Sloan Kettering Cancer Center  
New York, NY

Samuel A. Wells, Jr., M.D.

National Cancer Institute  
Bethesda, MD  
wells@mail.nih.gov

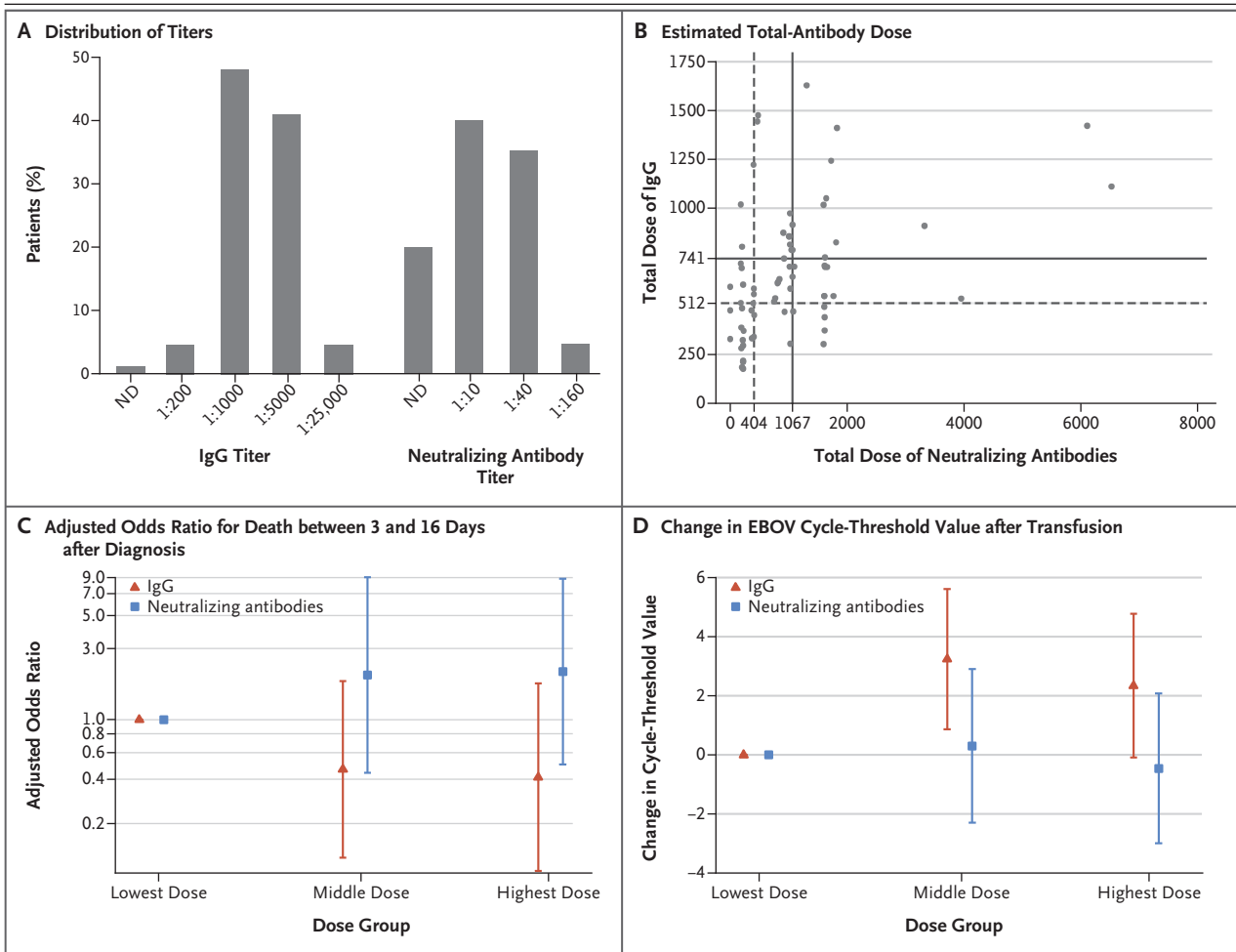
Since publication of their article, the authors report no further potential conflict of interest.

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## Efficacy of Convalescent Plasma in Relation to Dose of Ebola Virus Antibodies

**TO THE EDITOR:** We previously reported the results of a nonrandomized, controlled trial that compared survival among patients with Ebola

virus (EBOV) disease who were treated with convalescent plasma with survival among historical controls. Although no safety concerns were iden-



**Figure 1.** Titers of Total IgG and Neutralizing Antibodies against the Ebola Virus (EBOV) in Plasma from Convalescent Donors, Distribution of Total-Antibody Doses Given to Patients, Odds Ratios for Death between Days 3 and 16, and Changes in Cycle-Threshold Value after Transfusion.

Panel A shows the distribution of titers of total anti-EBOV IgG and neutralizing antibodies against EBOV in 85 donations from 58 convalescent donors whose plasma was used in the trial. ND denotes not detected. Panel B shows the distribution of the total dose of antibodies administered to patients with EBOV disease. To calculate the total dose that a patient received, the volume of the plasma unit was multiplied by the corresponding optical-density value from the enzyme-linked immunosorbent assay (for total anti-EBOV IgG) or antibody titer (for neutralizing antibodies); the sum of this measure for all plasma units that a patient received represented the estimated total-antibody dose. The total dose of neutralizing antibodies has been divided by a factor of 10. Infused plasma in which no antibodies were detected were allocated a zero dose in the estimation of the total dose. The dashed lines show the cutoff for the lowest-dose group of estimated antibody dose, and the corresponding solid lines show the cutoff for the middle-dose group (Spearman's  $\rho=0.425$ ;  $P<0.001$ ). Panel C shows the adjusted odds ratio for death between days 3 and 16 after diagnosis among 71 patients 16 years of age or older. The analysis used the lowest-dose group as the reference group, with adjustment for age and pretransfusion cycle-threshold value. I bars indicate 95% confidence intervals. Patients who died before day 3 after the diagnosis of EBOV disease were excluded.<sup>1,2</sup> In a test for association assuming a linear trend, after adjustment for age and cycle threshold,  $P=0.21$  for IgG and  $P=0.32$  for neutralizing antibodies. Panel D shows the change in EBOV cycle-threshold values from before to after transfusion among 83 patients 16 years of age or older. The analysis used the lowest-dose group as the reference group, with adjustment for age and pretransfusion cycle-threshold value. The cycle-threshold value is the number of cycles required for the fluorescence signal to cross the threshold for a positive result on the EBOV polymerase-chain-reaction assay; lower values indicate higher viral loads. I bars indicate 95% confidence intervals. More patients were included in this analysis than in the mortality analysis (see the Supplementary Appendix). In tests for heterogeneity between the dose groups (with adjustment for age and pretransfusion cycle-threshold value),  $P=0.02$  for IgG and  $P=0.82$  for neutralizing antibodies. In a test for a linear trend (with adjustment for age and pretransfusion cycle-threshold values),  $P=0.06$  for IgG and  $P=0.69$  for neutralizing antibodies.

tified, efficacy was not shown.<sup>1</sup> Notably, the levels of total anti-EBOV IgG and neutralizing antibodies in the infused plasma were unknown at the time of administration.<sup>1,2</sup> We now report on the association between the amount of total anti-EBOV IgG and neutralizing antibodies received and patient survival and on the changes in the amount of EBOV in their blood 24 hours after transfusion, expressed as the change in the cycle-threshold value in the polymerase-chain-reaction (PCR) analysis. The cycle-threshold value is the number of cycles required for the fluorescence signal to cross the threshold for a positive result on the EBOV PCR assay; lower values indicate higher viral loads.

The level of antibodies in the 85 donations was determined by means of an enzyme-linked immunosorbent assay (ELISA) and a plaque-neutralization assay (see the Supplementary Appendix, available with the full text of this letter at NEJM.org). ELISA titers for 94% of the donations were at least 1:1000. In the 50% plaque-neutralizing activity assay, most donations (75%) had a titer of 1:10 or 1:40, and only 4 (5%) had a titer of 1:160 (Fig. 1A). For each patient, a total-antibody dose was calculated by multiplying the volume of convalescent plasma infused by the EBOV antibody titer in the donation. The analysis was restricted to adults, because the dosing of convalescent plasma was done differently in children.

Patients were categorized into one of three equally sized groups on the basis of the estimated total-antibody dose (Fig. 1B). By chance, the pretransfusion cycle-threshold values were lowest in the highest-dose group for IgG and in the middle-dose group for neutralizing antibodies, and there was significant imbalance in the neutralizing-antibodies dose groups (see the Supplementary Appendix). Adjusting for age and pretransfusion cycle-threshold value, we observed lower mortality with higher IgG doses and higher mortality with higher doses of neutralizing antibodies, but neither of these associations was significant (Fig. 1C). The change in cycle-threshold values from before to after transfusion differed significantly according to IgG dose group ( $P=0.02$ ). However, there was little difference between the two higher-dose groups (Fig. 1D) and only weak evidence of a linear trend overall ( $P=0.06$ ). No association was apparent with the dose of neutralizing antibodies.

In conclusion, most patients received plasma with anti-EBOV IgG antibodies, but levels of neutralizing antibodies were low in many donations. The dose of IgG antibodies showed an association with larger increases in cycle-threshold values after transfusion but no significant association with mortality. Neither outcome showed an association with the estimated doses of neutralizing antibodies received. Further studies are needed to assess the effectiveness of an antibody dose higher than the doses used in this study, the antibody measure that best correlates with virologic and clinical outcomes, and the potential mechanism and clinical effect of viral clearance by anti-EBOV IgG antibodies.

Johan van Griensven, M.D., Ph.D.

Institute of Tropical Medicine  
Antwerp, Belgium  
jvangriensven@itg.be

Tansy Edwards, M.Math., M.Sc.

London School of Hygiene and Tropical Medicine  
London, United Kingdom

Sylvain Baize, Ph.D.

Institut Pasteur  
Lyon, France

for the Ebola-Tx Consortium

A complete list of collaborators in the Ebola-Tx Consortium is provided in the Supplementary Appendix, available with the full text of this letter at NEJM.org.

Supported by a grant (666094) from the European Union Horizon 2020 research and innovation program and by the Department of Economy, Science and Innovation of the Flemish government. The mobile plasma unit used in this trial was provided to Guinea by the Bill and Melinda Gates Foundation.

Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

This letter was published on November 14, 2016, at NEJM.org.

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DOI: 10.1056/NEJMc1609116

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