

IMPAIRED CD163 MEDIATED HEMOGLOBIN SCAVENGING

SEVERE TOXIC SYMPTOMS IN PATIENTS TREATED WITH GEMTUZUMAB OZOGAMICIN

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INTRODUCTION

Hemoglobin liberated to plasma during intravascular hemolyses is rapidly bound to haptoglobin. The hemoglobin-haptoglobin complexes undergo endocytosis through the monocyte/macrophage specific scavenger receptor for hemoglobin (CD163). This mechanism protects against oxidative and NO-scavenging adverse effects of free hemoglobin. CD33 is a membrane protein expressed on hematopoietic progenitor cells. Leukemic blast cells express the CD33 antigen in more than 90% of patients with acute myeloid leukemia (AML), which encouraged development of the CD33-targeted drug gemtuzumab ozogamicin (GO, Mylotarg™) (Figure 1).

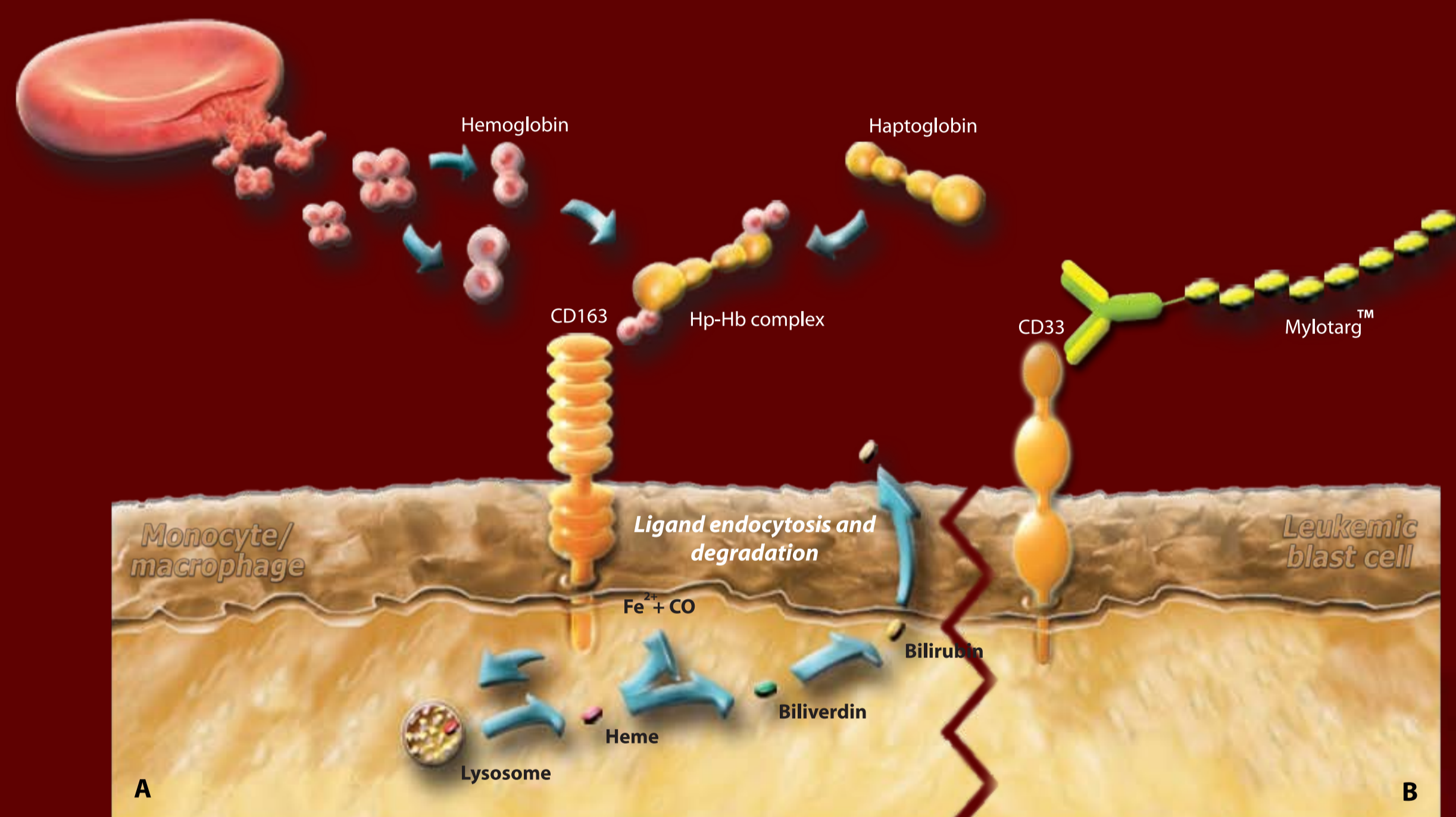


Figure 1: Overview of the receptor pathway for endocytosis of extracellular hemoglobin in complex haptoglobin (A) and the mechanism of CD33-directed therapy (B).

CD163 represent a pathway for uptake of extracellular haptoglobin-hemoglobin (Hp-Hb) complexes. The receptor is highly expressed in phagocytic macrophages, which are known to metabolize heme into bilirubin, iron, and carbon monoxide. The clearance Hp-Hb complexes in macrophages of the liver, spleen, and bone marrow contributes to the recycling of iron. Mylotarg is a recombinant humanized monoclonal CD33 antibody linked to calicheamicin, a potent cytotoxic agent. Mylotarg binds to CD33 resulting in the formation of a complex that is internalized. Upon internalization, the calicheamicin derivative is released inside the lysosomes of the myeloid cell. Calicheamicin derivative binds to DNA in the minor groove resulting in DNA double strand breaks which leads to cell death.

In this study, we present three cases of a novel syndrome of severe toxic symptoms during intravascular hemolysis occurring after treatment with CD33-directed therapy with the immunotoxin gemtuzumab ozogamicin. The syndrome is characterized by excessive accumulation of plasma hemoglobin despite high haptoglobin levels and it is to arise due to a GO-induced defect of monocytes/macrophages mediated hemoglobin scavenging.

AIM OF STUDY

To study the CD163 and CD33 coexpression in human peripheral blood cells and bone marrow, and investigate if the CD33-targeted chemotherapy leads to impaired CD163 mediated hemoglobin scavenging.

MATERIALS AND METHODS

Monocytic CD163 expression was investigated in patients with AML treated with gemtuzumab ozogamicin using multi-color flow cytometry.

CD163 and CD33 coexpression was investigated in peripheral blood using multi-color flow cytometry and in bone marrow using Immunofluorescence microscopy and immunohistochemical staining. Total RNA was isolated from peripheral blood and the CD163 mRNA expression was determined with reverse transcriptase real-time polymerase chain reaction. Plasma levels of soluble CD163 were measured using enzyme-linked immunosorbent assay.

RESULTS

A synchronous high free hemoglobin, haptoglobin, and low bilirubin after septicemia-induced intravascular hemolysis indicated abrogated clearance of haptoglobin-hemoglobin complexes.

A substantial fraction of CD14 positive monocytes expressing both CD33 and CD163 was identified in peripheral blood from healthy individuals. All CD163 positive cells stained positive for the CD33 antigen (Figure 2).

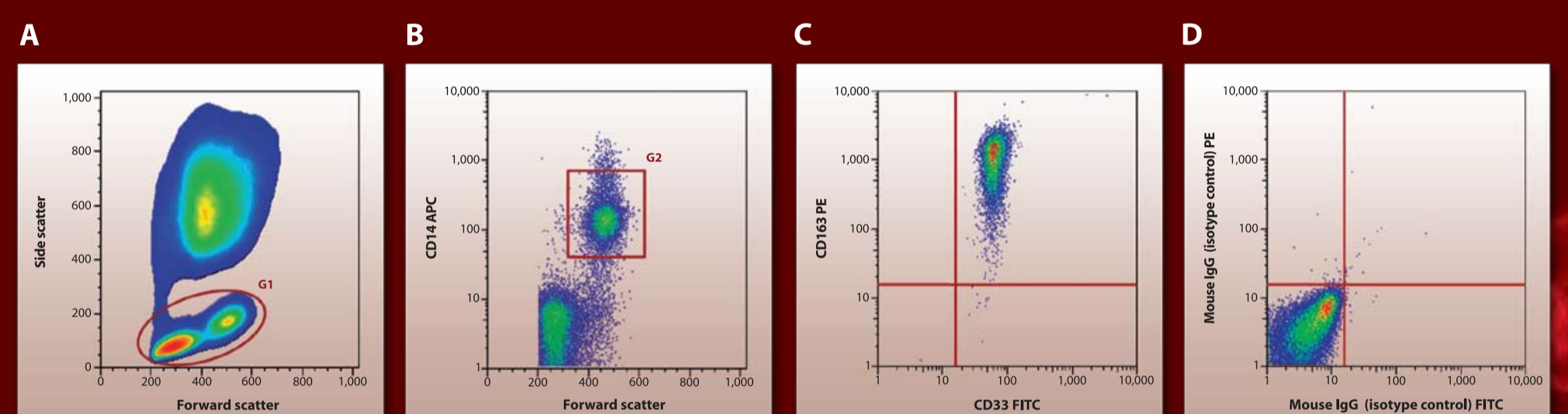


Figure 2: Flow cytometric analysis of CD33/CD163 coexpression in peripheral blood from healthy individuals. In a forward scatter [FSC] versus side scatter [SSC], the mononuclear cell cluster was gated (G1) (A). The gated cells were re-plotted with FSC versus CD14 APC (FL4), and a gate was set around CD14⁺ cells (G2; monocytes) (B). The gated cells were re-plotted in a pseudocolor dot-plot of CD33 FITC (FL1) versus CD163 PE (FL2) showing coexpression of CD33 and CD163 on CD14 positive monocytes (C). Isotype-matched PE- and FITC-conjugated mouse immunoglobulins were used as negative controls (D).

Double immunohistochemical staining (not shown) and immunofluorescence microscopy revealed that CD33 and CD163 are also coexpressed and colocalized on bone marrow macrophages from AML patients (Figure 3A).

During the hemolysis episode, an almost complete absence of CD163 positive cells in the circulation was demonstrated by flow cytometry (Figure 3B/C) and CD163 mRNA was not detectable (not shown).

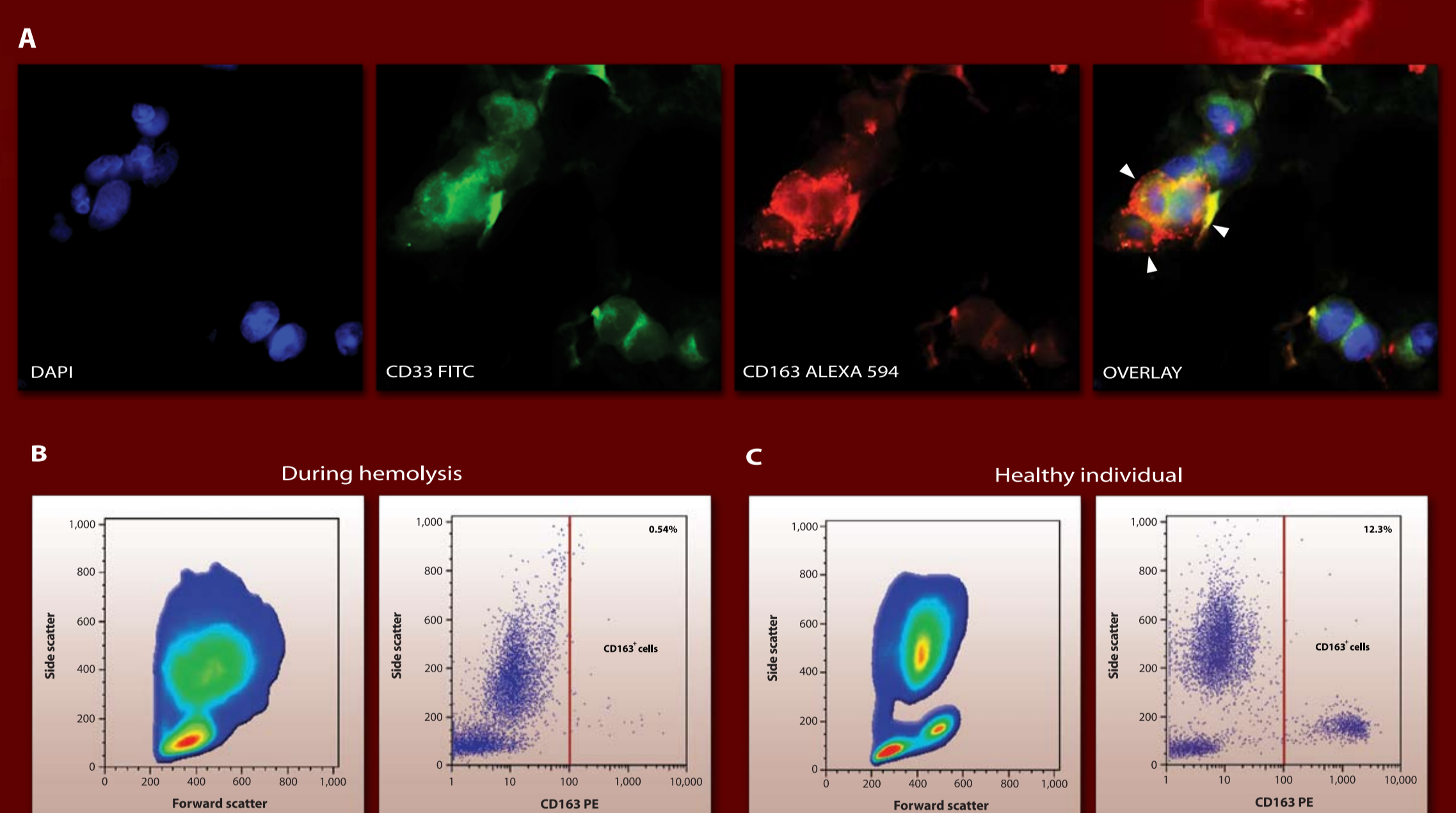


Figure 3: Bone marrow CD163/CD33 coexpression in patients with AML, and decreased peripheral blood CD163 expression in AML patients treated with gemtuzumab ozogamicin during intravascular hemolysis.

Immunofluorescence microscopy performed on formalin-fixed paraffin-embedded bone marrow sections from patient with relapse of AML before GO-treatment revealed the simultaneous presence of CD33 and CD163 on macrophages (A) (left panel: DAPI, blue immunofluorescence; left middle panel: FITC-conjugated anti-CD33, green immunofluorescence; right middle panel: biotin-conjugated anti-CD163/streptavidin-conjugated Alexa-Fluor[®] 594, red immunofluorescence; right panel: overlay, coexpression is revealed by yellow/orange merge color (marked with white arrowheads) (Original magnification $\times 100$, oil). Flow cytometric analysis of CD163 expression on peripheral blood leucocytes during the hemolysis episode in patient showed a complete depletion of monocytes and CD163 positive cells during the hemolysis episode (B), as compared to a healthy individual (C).

Further support for a severe elimination of CD163 positive cells was obtained by low levels of plasma soluble CD163 at the resolution of the hemolytic episode (1.60 $\mu\text{g/ml}$), excluding that the findings were due to a complete shedding of CD163. The levels increased to 7.40 $\mu\text{g/ml}$ during recovery (not shown).

CONCLUSION

The data presented in this study suggest that in cases of increased intravascular hemolysis, patients treated with GO are at risk of developing a severe novel syndrome caused by the exposition to the toxic effects of high levels of plasma hemoglobin. Increased attention to the syndrome may help initiate appropriate therapy at an earlier stage. Our findings emphasize the central role for CD163 in hemoglobin turn-over.