Liquid Biopsy Can Distinguish Recurrent GBM from Pseudoprogression and Radiation Necrosis After Concurrent Radiochemotherapy

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CNS 2016 ANNUAL MEETING San Diego CALIFORNIA SEPTEMBER 24-28, 2016

Abstract

Glioblastoma (GBM) is the most lethal primary brain tumor with a median survival of 15 months despite resection and concurrent radiochemotherapy. Among the many challenges in treating glioblastoma patients is the ability to differentiate pseudoprogression (PsP) and "radiation necrosis" (RN) from true recurrent GBM (rGBM). While PsP and RN occur in 15% to 30% of glioblastoma treated with concurrent radiochemotherapy, they are difficult to distinguish from recurrent GBM (rGBM) with magnetic resonance imaging (MRI). Thus, despite various MRI grading classifications, biopsy remains the gold standard. We and others have recently identified myeloid-derived suppressor cells (MDSC) in both tumor microenvironment and peripheral blood of GBM patients. Our recent studies also identified the MDSC-related protein VNN2 on CD14+ monocytes in various conditions

Methodology

Cell Isolation: PBMC were isolated by Ficoll-Histopaque centrifugation from 6mL of whole blood as described previously (Sugiyama et al, 2005). Samples were taken from patients and processed the same day (time-range 8h maximum after draw). CD14+ cells enriched by positive selection using CD14 magnetic beads (Miltenyi). Cells were electronically counted systematically using a BD C6 flow cytometer in order to reduce variability.Flow Cytometry Analysis: 50x104 CD14+ monocytes were surface-labeled with a combination of CD14-APC (Invitrogen) and HLA-DR-FITC (BD) or CD14-APC with VNN2-PE (MBL) in order to avoid compensation. VNN2-PE antibody was diluted 1:5 (2uL stock + 8uL of Wash Buffer). Cells were stained for 30 minutes at RT at dark into a 200uL total volume. Isotype-matched control antibodies were used.
Cells were resuspended in 200uL WB and analyzed using a C6 BD flow cytometer. Analysis was performed using Winlist software. The whole test took an average of 3h. Statistical Analysis. statistical significance was determined using Student t test.

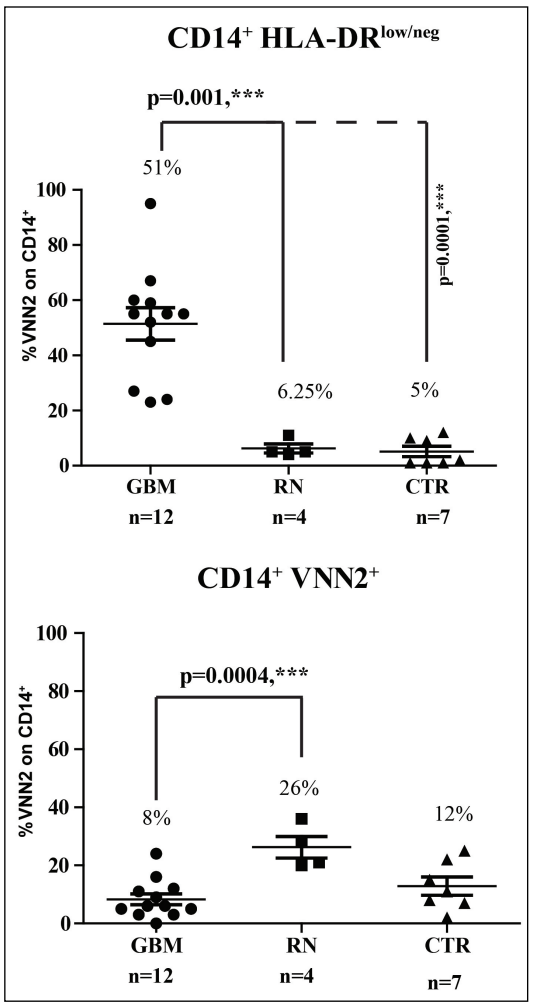

Figure 1. GBM patients have a higher number of CD14+ cells expressing low levels of HLA-DR (HLA-DRlow/neg) in their peripheral blood (i.e., Mo-MDSCs) compared to RN patients(Mean 51 %, n=12 vs. Mean 6.25%, n=4 respectively, p=0.001) with (B) an inverse proportion of CD14+ cells expressing lower VNN2 on GBM patients versus RN patients (Mean 8%, n=12 and Mean 26%, n=4 respectively, p=0.0004)

Results

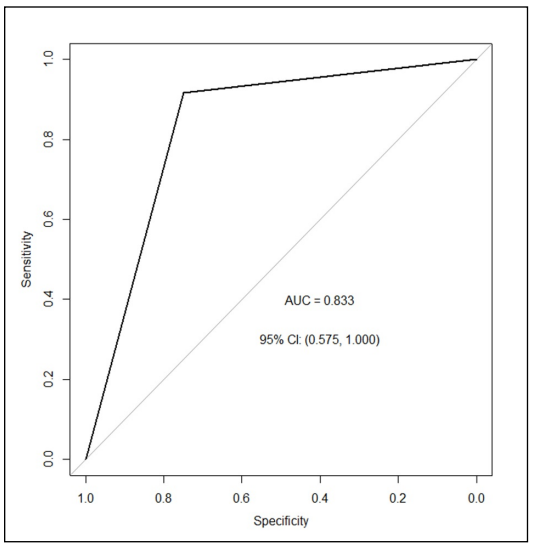

Figure 2. HLA-DR and VNN2 were categorized into binary variables using 20 as a cutoff value such that values <=20 got coded as "0" and values >20 got coded as "1". A receiver operating characteristic (ROC) curve was drawn and area under the curve (AUC) was calculated with its corresponding 95% confidence interval. This analysis was performed using R version 3.1.2 with the pROC library.

The combination percentage of HLA-DRlow/neg and VNN2+ cells among CD14+ monocytes from PBMC (DVI index) can be used to differentiate GBM from Radiation Necrosis patients

As shown in Figure 1A, GBM patients have a higher number of CD14+ cells expressing low levels of HLA-DR (HLA-DRlow/neg) in their peripheral blood (i.e., Mo-MDSCs) compared to RN patients(Mean 51 %, n=12 vs. Mean 6.25%, n=4 respectively, p=0.001) with (B) an inverse proportion of CD14+ cells expressing lower VNN2 on GBM patients versus RN patients (Mean 8%, n=12 and Mean 26%, n=4 respectively, p=0.0004). We unified both numbers by dividing the percentage of CD14+ HLA-DRlow/neg by the percentage of CD14+ VNN2+ cells into a unified hla-Dr Vnn2 Index (DVI) in order to differentiate GBM from RN accurately.

ROC analysis suggests the DVI index from this test is highly accurate and sensitive

We performed an Receiving Operating Characteristic (ROC) curve analysis of the data for GBM versus RN patients and as shown in Figure 2, the DVI index offers a high degree of accuracy in order to distinguish GMB or recurrent GBM patients from RN patients (Figure 2).

Conclusions

This novel, quick and inexpensive liquid biopsy test performed on human peripheral blood could replace invasive brain biopsy if preliminary results are confirmed in prospective studies currently underway.

Future directions

=>Complete a clinical trial validating this new diagnostic technique.
=>Follow-up GBM patients over time to test the predictive accuracy of this test.

Acknowledgments

I thank my PIs Dr. McCormick and Dr. Sloan.
This work was supported by the **Kimble Family Foundation** and the **Ferry Family Foundation**.

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