In the first study, collaborating investigators from Harvard Medical School and Biogen report on low-resolution single-molecule imaging by electron microscopy complemented by HDX to map the interface between FVIII and the VWF dimeric D’D3 fragment. The authors find considerable variability in the shape profiles of negatively stained electron microscopy images of the complex. Despite the implied flexibility in the D’D3 region, they note a consistent contribution of the C1 domain in mediating binding, but with some images implicating additional contacts with the C2 domain. These ideas are borne out by deuterium exchange with the polypeptide backbone impressively performed with coverage over the entire FVIII molecule both free and bound to the D’D3 dimer. They find a series of exchange hot spots in the C1 domain clustered in the vicinity of patient mutations that affect VWF binding. They also find areas of altered exchange in the C2 and A3 domains including the acidic a3 region. The major conclusions are that the C1 domain is the principal contributor to the binding of FVIII to the D’D3 fragment with additional or secondary contributions from the C2 and A3 domains including the a3 peptide.

In the second study, collaborating investigators at the University of Michigan address the same problem using low-resolution molecular envelopes reconstructed from single-particle electron microscopy studies. This second study not only addresses the interaction between the 2 proteins but also provides expanded insights into the disposition of both the dimeric D’D3 fragment as well as FVIII. Their image analyses reveal an antiparallel D3 dimer with the flexible D’ region projecting away from the dimer. The most extensive contacts are between the D’ region and one face of the C1 domain on FVIII extending toward the A3 domain and the a3 polypeptide region (see figure). The D3 dimer core extends across the base of the C1 and C2 domains with minimal contacts, interpreted to reflect additional weaker interactions. Rigid body fitting of the FVIII and D’ structures into the envelope suggest displacements of the C domains in comparison with their disposition in the x-ray structure. The principal conclusions from this study are that interaction between the C1 domain and the D’ region is the main determinant of the interaction between FVIII and VWF and that conformational plasticity in the interacting regions coordinates their high-affinity interaction.

The 2 studies reconcile the principal features associated with the binding of FVIII to VWF established by biochemical studies and the functional effects of C1 domain mutations in patients. The marked concordance in the conclusions arrived at by the 2 independent studies using different techniques is impressive and sheds new light on an important protein-protein interaction with major regulatory consequences for hemostasis.

The details of the interaction between FVIII and VWF uncovered in the 2 papers provide a major biochemical advance with the potential for revealing new strategies for translation to the clinic as the field increasingly turns to long-acting FVIII variants for the treatment of hemophilia.

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**CLINICAL TRIALS AND OBSERVATIONS**

Comment on Ceriani et al, page 950

**Quantitative FDG-PET: a new biomarker in PMBCL**

Michel Meignan  GROUPE HOSPITALIER MONDOR

In this issue of *Blood*, Ceriani et al introduce, in primary mediastinal B-cell lymphoma (PMBCL), a new prognostic factor measured on pretreatment 18F-fluorodeoxyglucose (18FDG)-positron emission tomography (PET)/computed tomography (CT): the total lesion glycolysis (TLG), which is an index of the glucose uptake by the total tumor burden. This paper is part of the International Extranodal Lymphoma Study Group (IELSG) 26 prospective study designed to evaluate the role of PET in the treatment of PMBCL.

PMBCL is a clinicopathologic entity of aggressive B-cell lymphoma that is clinically and biologically distinct from the other molecular subtypes of diffuse large B-cell lymphoma (DLBCL) and accounts for 2% to 4% of all non-Hodgkin lymphomas. It carries a relatively favorable prognosis in comparison with DLBCL as a result of patients’ younger age.
age and earlier stage at presentation. A 5-year survival rate >90% is reported in recent studies under aggressive immunochemotherapy with or without consolidation radiotherapy. Successful primary treatment is critical for PMBCL management because patients who failed or relapsed had a dismal outcome. A subset of patients develops an early refractory disease often around the completion of the first course of treatment, with a high failure rate after salvage treatment. Therefore, there is a need to identify these high-risk patients early when alternative therapeutic strategy, including more intensive treatment, could be considered. Prognosis factors are warranted for tailoring therapy and sparing the majority of young patients from the toxicity of these regimens. The international prognostic index (IPI) or age-adjusted IPI has not proven adequate. Other proposed clinical predictors of survival have not been validated prospectively, and biomarkers useful for risk stratification are now unavailable.

**18FDG** is an imaging biomarker surrogate of glucose metabolism. The degree of FDG uptake observed in lymphoma subtypes depends on the uptake of both tumor cells and cells from the microenvironment. It is impacted by the cross talk between them. In Hodgkin lymphoma (HL), FDG is chiefly a biomarker of the recruited accessory cells that account for >95% of the tumor, whereas in DLBCL, the uptake is mainly due to lymphoma cells. PMBCL is an FDG-avid lymphoma sharing some molecular characteristics with these 2 entities. The uptake is likely due to the lymphoma cells that are the main component of the tumor, but an uptake by the microenvironment cannot be excluded, as recent data have highlighted its important role in PMBCL.

18FDG-PET has been recognized as the best imaging tool for staging and response assessment in FDG-PET-avid lymphoma. Recently, Martelli et al reported (within the IELSG 26 study) a high prognostic value for end-of-treatment PET (eot-PET) in 125 PMBCL patients. The negative predictive value (NPV) was 98% for progression-free survival (PFS), which suggested that, on the basis of negative eot-PET, it could be possible to safely reduce the number of PMBCL patients to whom radiotherapy is given.

However, although the prognostic values of interim or eot-PET have been extensively studied in lymphoma, the role of quantitative data derived from baseline PET has been investigated less due to technical limitations. In HL, DLBCL, and T-cell lymphoma, prognostic values of baseline metabolic tumor volume (MTV) and TLG have been reported only on the basis of retrospective series. Ceriani et al report on the first study prospectively exploring the value of quantitative PET in aggressive lymphoma, focusing on 103 PMBCL patients. Maximum standardized uptake value (SUVmax), MTV, and TLG, all parameters dependent on lymphoma metabolism, measured on baseline FDG-PET/CT were negative prognostic factors for PFS and overall survival (OS). A higher risk of progression or deaths was associated with an MTV and TLG increase, but TLG was the strongest predictor of outcome independent of the stage of the disease. Long-term outcome was significantly better for patients with low TLG (less than the cutoff value) than for those with high TLG (greater than the cutoff value) with a 5-year OS of 100% vs 80% and 5-year PFS of 99% vs 64%, respectively. The method based on a percent thresholding of the tumor SUVmax was easy to use and reproducible (see figure). The technical difficulties of quantitative PET were minimized in PMBCL because tumor burden is mainly limited to a single bulky mass, which gives strength to the results of this study.

The high glycolytic activity observed in the high-risk patients might be explained by the increased proliferation of the malignant cells induced by the dysregulation of major signaling pathways and/or by the functional consequences for the microenvironment of their genetic alterations.

Although Ceriani et al report very high sensitivity (92%) for TLG with a 98% NPV for PFS, the positive predictive value (PPV)
was only 36%. Quantitative PET accurately identifies low-risk patients, but a better selection of high-risk patients is warranted to submit them to intensive treatment, and further studies are needed. To increase the risk stratification obtained with quantitative PET, it has been proposed to combine the PET baseline data with the PET response data or other clinical/biologic parameters, a method called integrative PET. In HL, a combination of baseline MTV with interim PET (iPET) has improved risk stratification, and iPET-negative patients could be stratified according to different risk molecular profiles. In this regard, Zucca et al reported in the same series of PMBCL patients that the combination of baseline TLG with end treatment PET more accurately identified patients at risk, with a PPV reaching 47% without a detrimental effect on NPV.

Because early stratification is preferred before the end of first-line therapy, other approaches could be investigated to define new prognostic models: baseline PET data can be combined with other clinical data or with molecular data such as the presence of XPO1 mutations recently reported as a recurrent alteration, which could be a biomarker of prognostic impact; they can also be combined with other PET data such as the heterogeneity of the SUV distribution in the tumor or with parameters obtained from other imaging techniques such as diffusion-weighted magnetic resonance imaging (MRI).

This stimulating study has opened an exciting field. It might be possible to use baseline quantitative FDG-PET to provide an earlier definition of a risk-adapted therapeutic strategy in PMBCL with this new imaging biomarker of tumor metabolism.

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Comment on Søgaard et al, page 957

Small clots with large impact

Jan Beyer-Westendorf1 and Walter Ageno2 1UNIVERSITY HOSPITAL “CARL GUSTAV CARUS”; 2UNIVERSITY OF INSUBRIA

In this issue of Blood, Kirstine Søgaard and colleagues report on the relevance of splanchnic vein thrombosis (SVT) as a marker of occult malignant disease.

SVT is an uncommon but potentially life-threatening disease. It can affect the portal vein, mesenteric veins, splenic vein, or suprahepatic veins in Budd-Chiari syndrome, with symptoms varying from asymptomatic cases detected during imaging procedures to symptoms of acute abdomen or active gastrointestinal bleeding. Many SVT events are caused by underlying clinical conditions such as liver cirrhosis, pancreatitis, inflammatory bowel disease, or abdominal surgery.

However, SVT is also commonly associated with solid abdominal cancer, Philadelphia-negative myeloproliferative neoplasms, or JAK2V617F mutation. Although most SVT events occur during cancer therapy or are incidentally detected during restaging of malignancies, the diagnosis of SVT may also precede the diagnosis of these malignant conditions. It was with this in mind that the authors set out to use large nationwide linked health care databases in Denmark to identify patients with a newly diagnosed SVT to study the prognostic relevance of SVT for later cancer occurrence and survival.

For us, this paper contributes outstandingly to our knowledge on the relevance of SVT for the following reasons:

- The applied methodology is rigorous, because it uses nationwide linked health care databases with unique patient identifiers. These databases cover not only diagnoses and hospitalizations but also comorbidities, treatments, and outcomes, including details on mortality. Such a database system has never been used to study the prognostic relevance of SVT. With this methodology, absolute risks and standardized incidence ratios for developing cancer in the years after SVT diagnosis could be calculated.

- The same methodology allowed a matched-pair comparison with cancer patients without SVT and, therefore, a detailed assessment of the contribution of SVT to short-term mortality in cancer patients.

The main findings of this paper were:

1. SVT is a marker of occult solid tumors, such as liver and pancreatic cancer, and not only of myeloproliferative neoplasms, as previously shown by a number of studies.
2. SVT is a prognostic factor for short-term survival in patients diagnosed with liver and pancreatic cancer.

Readers may feel that these findings are hardly surprising, since it is known that patients with unprovoked venous thromboembolism (VTE) in general are at higher risk of occult malignant disease. Furthermore, it may be regarded as standard