18F-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography Enables the Detection of Recurrent Same-Site Deep Vein Thrombosis by Illuminating Recently Formed, Neutrophil-Rich Thrombus

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Background—Accurate detection of recurrent same-site deep vein thrombosis (DVT) is a challenging clinical problem. Because DVT formation and resolution are associated with a preponderance of inflammatory cells, we investigated whether noninvasive 18F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) imaging could identify inflamed, recently formed thrombi and thereby improve the diagnosis of recurrent DVT.

Methods and Results—We established a stasis-induced DVT model in murine jugular veins and also a novel model of recurrent stasis DVT in mice. C57BL/6 mice (n=35) underwent ligation of the jugular vein to induce stasis DVT. FDG-PET/computed tomography (CT) was performed at DVT time points of day 2, 4, 7, 14, or 2+16 (same-site recurrent DVT at day 2 overlaying a primary DVT at day 16). Antibody-based neutrophil depletion was performed in a subset of mice before DVT formation and FDG-PET/CT. In a clinical study, 38 patients with lower extremity DVT or controls undergoing FDG-PET were analyzed. Stasis DVT demonstrated that the highest FDG signal occurred at day 2, followed by a time-dependent decrease (P<0.05). Histological analyses demonstrated that thrombus neutrophils (P<0.01), but not macrophages, correlated with thrombus PET signal intensity. Neutrophil depletion decreased FDG signals in day 2 DVT in comparison with controls (P=0.03). Recurrent DVT demonstrated significantly higher FDG uptake than organized day 14 DVT (P=0.03). The FDG DVT signal in patients also exhibited a time-dependent decrease (P<0.01).

Conclusions—Noninvasive FDG-PET/CT identifies neutrophil-dependent thrombus inflammation in murine DVT, and demonstrates a time-dependent signal decrease in both murine and clinical DVT. FDG-PET/CT may offer a molecular imaging strategy to accurately diagnose recurrent DVT. (Circulation. 2014;130:1044-1052.)

Key Words: fluorodeoxyglucose F18 ▪ inflammation ▪ neutrophils ▪ positron-emission tomography ▪ venous thrombosis

Deep vein thrombosis (DVT) and subsequent pulmonary embolism is a major cause of cardiovascular death. The incidence of DVT in industrialized countries is 1 to 3 individuals per 1000 per year. Furthermore, the risk of recurrent DVT is up to 10% per year in patients with idiopathic or unprovoked DVT. In addition, after DVT treatment, patients may experience recurrent leg pain because of a recurrent DVT, the postthrombotic syndrome, or other etiologies. Failure to diagnose a recurrent DVT places the patient at risk of fatal pulmonary embolism. Therefore the accurate diagnosis of a recurrent DVT is critical to preserve health, and to justify prolonged, potentially life-long, anticoagulation and its attendant bleeding risks, as well. Unfortunately, the diagnosis of a recurrent DVT often poses a significant diagnostic challenge for anatomic imaging methods such as duplex ultrasonography, computed tomography (CT) venography, or magnetic resonance venography, because a prior residual thrombus confounds the diagnosis of acute superimposed on remote thrombus. Thus, accurate approaches to identify recurrent DVT are urgently needed.

Clinical Perspective on p 1052

DVT formation and resolution are time-dependent inflammatory processes that involve neutrophils and macrophages.
Because 18F-fluorodeoxyglucose (FDG) uptake is upregulated in lesions containing proinflammatory myeloid cells such as neutrophils, macrophages, we hypothesized that FDG-poson emission tomography (PET)-computed tomography (CT) could allow noninvasive imaging of DVT-induced inflammation, and allow the identification of recurrent DVT even in sites with preexisting older thrombi. In this study, we systematically investigated the imaging profile of FDG-PET in murine DVT, and then explored cellular mechanisms of FDG uptake in DVT, focusing on thrombus neutrophils and macrophages. Then, we developed and validated a novel animal model of recurrent stasis DVT to investigate whether FDG-PET could specifically identify recurrent DVT in the same site as the primary DVT. Additionally, in a blinded clinical study, we investigated the temporal profile of FDG uptake in DVT patients undergoing FDG-PET.

**Methods**

**Mouse Model of Stasis-Induced DVT in the Jugular Vein**

Animal studies were approved by the MGH Subcommittee on Research Animal Care. Male C57BL/6 mice (14–20 weeks) were anesthetized by using a mixture of intraperitoneal ketamine/xylazine. The right main jugular vein and a reliably present large side branch were surgically ligated with 6-0 nylon sutures to induce stasis DVT, analogous to murine inferior vena cava (IVC) stasis DVT models. As a sham control, the left jugular vein was surgically exposed and tied loosely without constriction (Figure 1A). At various time points after ligation from day 2 up to day 16, PET/CT imaging was performed, followed by euthanization and ex vivo analyses (n=4–6 at each time point). A subset of mice (n=3) underwent 3–time point serial PET/CT imaging and were euthanized after the final imaging time point (Figure I in the online-only Data Supplement). For the recurrent DVT model (n=6), we removed the suture of the ligation at day 2 to allow recanalization of thrombosed jugular vein, followed by religation 12 days later to induce recurrent same-site stasis-induced DVT (Figure I in the online-only Data Supplement). We also performed complete ligation of the contralateral jugular vein at day 2 to enhance recanalization of the first DVT, and to compare a fresh recurrent DVT and an older DVT in the same mouse.

**PET/CT Imaging**

Following an overnight fast, mice underwent tail vein injection of 15 to 25 μCi/g 18F-FDG (PETNET Solutions, Woburn, MA). One hour later, micro-PET/CT imaging was performed by using a small animal scanner (Inveon, Siemens Medical Solutions, Inc, Malvern, PA). CT imaging preceded PET imaging, with acquisition of 360 cone beam projections by using a source power and current of 80 keV and 500 mA, respectively. Mice were injected with an iodinated contrast agent (Isodev-370) at 20 μL/min during the CT scan. Projections were reconstructed into 3-dimensional volumes containing 512×512×768 voxels with the dimension of 0.11×0.11×0.11 mm (Amira, San Diego, CA). The PET image voxel size was 0.7979×0.8610×0.8610 mm. Data were calculated as mean and maximum standardized uptake values (SUVs) and target-to-background ratio (TBR; DVT/sham).

**Ex Vivo Gamma Counting and Histopathology**

See online-only Data Supplement for full details. After euthanization, mice were perfused with 0.9% saline via the left ventricle. Radioactivity of excised jugular veins was measured by a gamma radiation counter (Wizard, PerkinElmer). In a subset of resected DVT (day 2 time point, n=5), the vein wall and thrombus were gently separated followed by gamma radioactivity measurements. Next, jugular veins were fixed overnight with paraformaldehyde and embedded in optimal cutting temperature compound (Sakura Finetek, Torrance, CA). Serial 6-μm cryostat sections were obtained for hematoxylin-eosin, Carstairs fibrin staining, Masson trichrome, and immunohistochemistry. The number of thrombus neutrophils and macrophages per 5 high-power fields (1000×) was quantified as previously reported.

**Protein Extraction and Immunoblotting**

See online-only Data Supplement.

**Clinical FDG-PET Study**

See online-only Data Supplement for full details. Access of patient records and analysis of patient data were approved by the Partners Institutional Review Board. Participants for the clinical imaging sub-study were consecutively identified from a database of patients who had undergone 18F-FDG-PET/CT imaging for oncological evaluation at Massachusetts General Hospital. From a clinical database of 437 patients who underwent PET/CT imaging and also had a clinical diagnosis of DVT, we consecutively identified 19 patients with iliolumbar
DVT and whose FDG-PET imaging was performed within 6 months after the onset of DVT. Matched control patients were then identified for each individual with a DVT by age, sex, malignancy status, chemotherapy exposure, and steroid immunosuppressive therapy within 6 months. An individual region-of-interest was placed around the area just superior to the popliteal vein and extended to the inferior of the iliac bifurcation to obtain a maximum standardized uptake value (SUVmax). The average venous blood uptake of the right atrium was used to derive a TBR. Patients were then analyzed to assess the time-dependent changes in FDG uptake in DVT and corresponding vein of matched control patients without a DVT.

Statistics
See online-only Data Supplement for full details. For animal data, results are expressed as median (25%–75% quartiles). Statistical comparisons between 2 groups were evaluated by the Mann-Whitney U test, and by the Kruskal-Wallis test for multiple groups followed by Dunn post test. For comparison between 2 groups within the same animal, the Wilcoxon matched-pairs signed rank test was used. Continuous variables at multiple time points were compared by the Friedman test. FDG uptake measurements were correlated with histological findings by use of the 2-tailed Spearman method. Statistical comparisons were performed with GraphPad Prism (La Jolla, CA). Mice that did not develop jugular DVT histologically negative, n=3) were excluded from analyses. For clinical data, values are expressed as mean ± standard error of the mean. The Wilcoxon signed rank test was used for single comparisons, and, after the normality of distribution was confirmed, linear regression analysis was used to determine the association between FDG uptake and DVT age (SPSS 22, IBM, Chicago, IL). A value of P<0.05 was considered statistically significant.

Results
Development of a Murine Stasis-Induced DVT Model in the Jugular Vein Suitable for FDG-PET Imaging
The surgical ligation-induced stasis model of murine DVT is well established in the IVC.14,15,17 However, preliminary experiments with FDG-PET imaging of acute IVC DVT did not allow thrombus detection owing to the high FDG background in the kidneys and spine, and in the surrounding bowel, an organ with high glucose use, as well. Therefore, we established a new murine stasis-induced DVT model in the jugular vein, an area of lower background FDG uptake and a common site of clinical DVT.5 Jugular DVTs were readily detected as obstructions on CT venography, in contrast to sham-operated contralateral jugular veins (Figure 1A and 1B). Histological analyses confirmed fibrin, red blood cells, and white blood cells, similar to the ligation IVC stasis model (Figure 1C through 1E).14,17,20

Noninvasive Imaging of DVT Inflammation by FDG-PET/CT
In vivo 18F-FDG PET/CT imaging was performed from day 2 up to day 16 after jugular vein ligation. Elevated FDG signal was observed in the right thrombosed jugular vein, with significantly less signal detected in the sham-operated jugular vein (Figure 2A, FDG-PET). Thrombosed jugular veins induced a filling defect on CT venography (Figure 2B, CT) that colocalized well with FDG-PET signals (Figure 2C, PET/CT).

18F-FDG Accumulation Diminishes Over Time in Experimental DVT
To determine whether the FDG-PET signal was modulated by DVT age, we analyzed DVT signals as a function of time after jugular vein ligation. We observed a significant decrease in the 18F-FDG DVT signal over time (SUV (median [quartiles])=1.98 [1.87–2.70] day 2, 1.20 [1.04–1.30] day 4, 0.88 [0.82–0.96] day 7, 0.68 [0.57–1.22] day 14, P<0.001; Figure 3A). The DVT SUVmax and TBR values were highest at day 2 and decreased thereafter (SUVmax=2.31 [2.20–3.23] day 2, 1.35 [1.17–1.58] day 4, 1.07 [0.92–1.27] day 7, 0.93 [0.66–1.54] day 14, P<0.01; TBR=1.85 [1.79–2.09] day 2, 1.49 [1.45–1.79] day 4, 1.19 [1.10–1.32] day 7, 1.13 [1.08–1.23] day 14, P=0.002, Figure 3B and 3C). Gamma radiation counts of resected DVT demonstrated similar findings, with a time-dependent decrease in radioactivity (% injected dose per gram of tissue=7.81 [6.47–9.21]% day 2, 7.72 [7.14–13.1]% day 4, 6.47 [5.25–9.36]% day 7, 3.04 [2.48–5.44]% day 14, versus 0.735 [0.458–1.75]% [pooled sham], P<0.0001, Figure 3D).

Three mice underwent serial PET/CT imaging at day 2, 4, and 14 after the ligation. Serial imaging revealed a time-dependent decrease in the FDG signal in individual DVT (P=0.03, Figure 3E and 3F).

Neutrophil Appearance Associates With FDG Uptake in DVT
We compared 18F-FDG uptake measurements and histological profiles at the study time points of 2, 4, 7, and 14 days after induction of stasis DVT. Neutrophils infiltrated DVT abundantly at the earlier time points of day 2 and 4, with fewer neutrophils observed at day 7, and minimal neutrophils at day 14 (Figure 4). Thrombus macrophages were evident at day 7 and resided in the outer DVT edge (Figure 4). These histological findings in our developed jugular DVT stasis ligation model recapitulate
both the IVC full-stasis DVT and the partial-flow IVC DVT models.14,20 The number of neutrophils in DVT correlated with the FDG DVT uptake (SUV, \( r = 0.41, P = 0.004; \) TBR, \( r = 0.62, P < 0.001 \), Figure 5A). The macrophage FDG relationships were analyzed in day 7 and day 14 DVT, because day 2 and day 4 DVT showed few macrophages and also substantial confounding neutrophils. In the day 7 and 14 cohort, macrophages were not significantly associated with the FDG DVT signal (SUV, \( r = –0.15, P = 0.47; \) TBR, \( r = 0.26, P = 0.23 \), Figure 5B). To avoid confounding neutrophil-based FDG signal at day 7, we further assessed only the day 14 macrophage association with FDG signals. This correlation remained nonsignificant (SUV, \( r = 0.04, P = 0.91; \) TBR, \( r = 0.30, P = 0.37 \), Figure 5C).

To further examine the relationship of FDG-PET and thrombus inflammation, we assessed the protein expression of matrix metalloproteinase-9, a key inflammatory mediator of DVT.8 Immunoblot analyses showed a time-dependent decrease of matrix metalloproteinase-9 expression in DVT (Figure II A and IIB in the online-only Data Supplement, \( P = 0.01 \)), similar to the in vivo FDG signals.

To evaluate the relative contributions of FDG signal from the thrombus and from the vein wall, we performed ex vivo gamma radiation measurements after carefully separating the thrombus and the vein wall components (day 2 DVT, \( n = 5 \)). We found that 66.7±5.9% of the radioactivity localized in thrombus and 33.3±5.9% in the vein wall. Immunohistological images showed that neutrophils (NIMP-R positive area) were localized to thrombi, with the remaining 14% localized to the vein wall. These data indicate that the majority of the FDG signal arises from thrombus itself.

Figure 3. Time-course quantitative changes of FDG signal in DVT. Standard uptake value (SUV; A), SUVmax (B), target-to-background ratio (TBR; C), and % injected dose per gram of tissue (%IDGT; D) showed a similar trend (\( P < 0.05 \)) in that values were highest at day 2 and significantly decreased over time (4–5 mice per group). E and F, Subset of mice underwent serial PET imaging at day 2, 7, and 14. FDG accumulation in DVT (yellow arrow, sham control white arrow) demonstrated a time-dependent significant decrease (\( P = 0.03 \)). *\( P < 0.05; **P < 0.01; ***P < 0.001 \). Box-and-whisker plot: Middle line represents median value, box indicates interquartile range (25th–75th percentiles), and range bars show maximum and minimum. DVT indicates deep vein thrombosis; FDG, \(^{18}\)F-fluorodeoxyglucose; and PET, positron emission tomography.
Neutropenia Markedly Reduces FDG-PET Signal Generation in DVT

Histological analyses above demonstrated that thrombus neutrophils, but not macrophages, provided the basis for elevated FDG signals in DVT. To further determine whether neutrophils could directly modulate the FDG signal in DVT, we induced systemic neutropenia in a subgroup of mice before DVT induction. Pretreatment with an antineutrophil antibody decreased circulating neutrophils in day 2 DVT mice (0.6 [0.2–0.9] × 10^3/μL versus control day 2 DVT mice, 1.7 [1.2–2.1] × 10^3/μL, P=0.03). On day 2, imaging demonstrated a significant decrease in FDG-DVT signal in neutropenic mice in comparison with normal mice (SUV=1.15 [1.06–1.51] versus 1.98 [1.87–2.70], P=0.03, Figure 6). Histological assessment confirmed diminished neutrophil accumulation in DVT (neutropenia 21.0 [14.0–59.0] versus control 102 [86.0–126], number of neutrophils per 5 high-power-fields, P<0.001).

Establishment of Novel Animal Model of Recurrent DVT

To our knowledge, this is the first report demonstrating an animal model of recurrent DVT. The conventional stasis-induced DVT model is not suitable for inducing recurrent DVT because of the absence of adequate blood flow and blood volume after complete ligation,14 precluding generation of a second, same-location, stasis-induced DVT. To create an environment suitable for a second stasis DVT, at day 2 we deligated (cut the suture) the initial jugular vein ligation to spur partial thrombus recanalization and restoration of blood flow. In addition we ligated the contralateral jugular vein to induce an occlusive stasis DVT. Twelve days later (at day 14), the deligated vein was then religated at the same location (Figure 7A). This procedure successfully generated a second new venous thrombus at day 2 directly overlaying the 16-day-old primary thrombus. Histological staining clearly distinguished the second thrombus at day 2 (increased fibrin, increased neutrophils, and less collagen) from the organized primary thrombi at day 16 (Figure 7B through 7D). The vein wall also exhibited DVT-induced scarring (increased thickness and collagen-rich), which is observed in patients with postthrombotic syndrome.21 A number of neutrophils were present in day 2 recurrent DVT, whereas macrophages were abundant in day 16 DVT, similar to day 14 DVT (Figure 7E and 7F).

FDG-PET/CT Enables the Identification of Recurrent, Same-Site DVT

Because our data revealed that FDG signal is neutrophil dependent, we examined whether FDG-PET could identify recently formed, neutrophil-rich recurrent DVT in the same site as the primary DVT. As shown in Figure 7G through 7I, recurrent DVT was successfully identified by FDG-PET/CT. FDG signal from recurrent DVT (recurrent DVT at day 2+primary DVT at day 16) was significantly higher than old day 14 DVT (SUV; 1.65 [1.42–1.83] versus 0.962 [0.892–1.07], SUVmax; 2.01 [1.63–2.17] versus 1.06 [0.973–1.16], P=0.03, respectively, Figure 7J and 7K). Ex vivo gamma radiation measurement also showed a significant increase in the activity of recurrent DVT (day 2+16) in comparison with older DVT at day 14 (% injected dose per gram of tissue=20.46 [16.4–26.3]% versus 4.60 [2.46–7.75]%, P=0.02, Figure 7L).
18F-FDG Activity in DVT Decreases Over Time in Patients

We retrospectively analyzed 38 individuals who underwent PET/CT scanning for clinical indications: 19 patients with DVT (10/9 male/female; mean age, 64.1 years) and 19 matched control patients (Table I in the online-only Data Supplement). We observed a significantly increased signal within recently formed DVT (Figure 8A and 8B). Across the entire population, the FDG signal in DVT was significantly greater than in the matched vein of controls (SUVmax 1.87±0.15 versus 1.32±0.10, \( P = 0.02 \); TBR 1.62±0.19 versus 1.10±0.05, \( P = 0.01 \)). Furthermore, we observed a time-dependent decrease in the FDG signal within DVT. When the DVT age was stratified by tertiles, both the SUVmax and TBR of DVT diminished over time (\( P = 0.002 \) for SUVmax, \( P = 0.004 \) for TBR, Figure 8C and 8D). The relationship between DVT age (by tertiles) and DVT FDG uptake (either as SUVmax or TBR) remained significant after multivariable adjustments for: demographic factors (TBR: \( \beta = -0.209, P = 0.007 \); SUVmax: \( \beta = -0.233, P = 0.003 \)); factors that could impact systemic inflammation (TBR: \( \beta = -0.228, P = 0.002 \); SUVmax: \( \beta = -0.237, P = 0.003 \)); and oncological history (TBR: \( \beta = -0.225, P = 0.003 \); SUVmax: \( \beta = -0.245, P = 0.001 \)).

Discussion

In this experimental and clinical study, we demonstrate that the intense inflammatory response produced by DVT can be systematically imaged, serially assessed, and quantified with noninvasive PET/CT. The FDG inflammatory signal exhibited a time-dependent and neutrophil-dependent decay in murine DVT. We also established that FDG-PET/CT could differentiate recurrent DVT from primary DVT by the use of a novel animal model of recurrent DVT. Furthermore, in a clinical study, we observed a similar time-dependent decrease in the FDG-PET signal in human DVT. The overall findings demonstrate...
that FDG-PET/CT provides an accurate approach to assess neutrophil-dependent and age-dependent DVT inflammation, and can specifically diagnose recurrent DVT.

Recurrent DVT occurs up to 10% per year in patients with unprovoked DVT\(^1\) and is a highly morbid condition, increasing the risk of the postthrombotic syndrome, pulmonary embolism, and death.\(^2\,22\,23\) Accurate diagnosis of recurrent DVT is therefore important in initiating timely anticoagulant therapy, determining the duration of treatment, and recognizing whether a failure of anticoagulation has occurred, with implications for considering lower extremity venography to reduce the risk of pulmonary embolism.\(^2\) However, the diagnosis of a recurrent ipsilateral same-site DVT, defined as a new DVT occurring in the same location as a previous DVT, poses a significant diagnostic challenge. Although recurrent DVT is common as an end point in observational and therapeutic clinical venous thromboembolism trials,\(^3\) there is no gold standard for its diagnosis, including ultrasound, MRI, CT, or venography.\(^5\,6\) Venous compression ultrasound, the gold standard for initial DVT diagnosis,\(^25\) is limited in cases of recurrent DVT where residual thrombus or vein wall scarring is present after the initial DVT. The current findings suggestive of prior DVT on duplex ultrasonography include retraction of the vein, the presence of collateral veins, and recanalization. These signs are often subtle and are frequently absent. Diagnosis of an acute DVT in the milieu of a prior DVT is particularly difficult, especially if large changes in thrombus length or compressed vein diameter are not apparent.\(^5\,6\) The situation is even more complicated if previous ultrasound images are unavailable, and some patients may require invasive venography to attempt to establish a diagnosis, which is also limited in its accuracy.\(^5\) In the past, studies have attempted to distinguish acute from organized thrombi with ultrasonography,\(^26\) MRI,\(^27\,28\) and single-photon emission CT imaging.\(^29\,30\) However, no established imaging technique is routinely used in the clinic.\(^5\) The current findings herein demonstrate that FDG-PET/CT can distinguish newly formed, neutrophil-rich thrombus from older thrombus and can specifically identify recurrent DVT.

Whereas a single case report suggested the potential for FDG-PET/CT to detect recurrent DVT, the authors did not specify if DVT recurred in the same location as the original DVT, nor systematically assess the age of the thrombus, nor obtain histological assessment.\(^31\) In addition, although other clinical reports have demonstrated that FDG-PET can identify DVT (but not specifically recurrent DVT),\(^32\,33\) our experimental results extend these previous studies, because we precisely controlled the DVT age and timing of subsequent FDG-PET/CT imaging studies, performed quantitative ex vivo radioactivity, and detailed histology assessments to corroborate in vivo FDG-PET signals, and induced neutropenia to modulate the FDG-PET signal.

Because we established that the FDG DVT signal is time dependent, FDG-PET offers a noninvasive approach to assess DVT age in vivo. This finding has implications for fibrinolytic therapy of DVT using catheter-directed therapy or pharmacomechanical therapy, two emerging clinical strategies used to treat large iliofemoral DVT.\(^34\,35\) This is because intravascular-based fibrinolysis of DVT appears more effective for earlier stage thrombi that are <10 days old.\(^36\) Because the precise age of DVT is not often possible to assess clinically due to the insidious onset of flow-limiting DVT, FDG-PET assessment of DVT age might help predict the success of catheter-directed therapy or pharmacomechanical therapy.

In this study, the observation that the inflammatory FDG signal was more closely associated with neutrophils than macrophages in DVT is not unexpected. FDG uptake occurs in cells exhibiting increased glycolysis, which is higher in myeloid cells, particularly activated macrophages.\(^37\,38\) We determined that the FDG-PET signal was neutrophil dependent, and accordingly higher in early, neutrophil-rich DVT. Subsequently, in resolving subacute DVT, we hypothesized that infiltrating macrophages might be of an M2-polarized (reparative) phenotype, because M2-macrophages do not exhibit upregulated glucose transporter protein type 1 (GLUT-1) expression needed to concentrate FDG.\(^12\)\(^,39\) Histological analyses supported this hypothesis, because macrophages within day 14 DVT in fact displayed little GLUT-1 expression and little iNOS expression, an M1 marker (Figure III in the online-only Data Supplement). This is in distinction to chronic inflammatory conditions such as in atherosclerosis, where macrophages account for the FDG signal.\(^13\,37\)\(^,40\,41\) and demonstrate an M1-polarized (proinflammatory) and GLUT1-upregulated phenotype.\(^12\) Moreover, the neutrophil-based FDG findings in DVT are similar to studies of other acute, neutrophil-rich lesions such as acute lung injury, where neutrophils but not macrophages serve as the main cellular source of FDG uptake.\(^9\,11\) Because there was a mild FDG signal in day 14 DVT without neutrophils, other nonmacrophage cells that may have accumulated FDG include activated myofibroblasts, which are present in the remodeling vein wall after DVT\(^7\,44\) and ingest FDG in experimental arthritis models.\(^45\) Another consideration regarding lower FDG signal in the later-stage DVT could be due to greater thrombus organization, limiting access of FDG to interior thrombus cells.

Limitations of this study are present. Because stasis-induced DVT was generated by surgical ligation of the jugular vein, surgery-induced inflammation is inevitable, especially at day 2, resulting in FDG uptake at the wound-healing region around the DVT and likely in the venous wall. Whereas experiments demonstrate that the majority of FDG signal arises from the thrombus, the vein wall also contributes modestly to the FDG signal. Because the ligation suture is necessary to induce stasis thrombi, we cannot precisely resolve intrinsic vein wall inflammation that occurs during DVT resolution from ligation-induced injury. Second, our clinical cohort did not contain any patients with a recurrent DVT who underwent FDG-PET imaging. Third, the clinical cohort was derived from individuals undergoing imaging for oncological indications, and was a retrospective study. Accordingly, generalization of the findings may be constrained, and prospective clinical FDG-PET studies of recurrent DVT and serial DVT imaging will be needed to validate our findings. Finally, although it is known that\(^19\)\(^-\)\(^21\)FDG uptake is upregulated in lesions containing proinflammatory myeloid cells such as neutrophils\(^8\)\,\(^9\)\,\(^11\) and macrophages,\(^12\)\,\(^13\) it was not possible to perform histological analysis of clinical DVTs in this study. Future FDG-PET DVT clinical studies that include a DVT biopsy will be able to...
elucidate specific inflammatory cells underlying FDG uptake in human DVTs.

Clinical Implications

Our clinical retrospective study also demonstrated a time-dependent decrease in the FDG signal within DVT, extending the main experimental findings from the animal study. DVT showed significantly greater FDG signal than the matched vein of control patients. By analyzing greater numbers of DVT patients and with clot ages up to 21 weeks, our findings extend a smaller study that was restricted to ≤10-week-old thrombi.32

The findings of this study support the concept that FDG-PET imaging might be useful for determining the inflammatory activity of DVT in living subjects. It is possible that PET/CT could be used when there is uncertainty regarding whether recurrent DVT exists, or to what degree there is a substantial inflammatory component to an initial DVT, which might also inform the risk of the postthrombotic syndrome.66–67 Ultimately, prospective clinical studies will need to be conducted to evaluate if FDG-PET imaging of recurrent DVT provides additive diagnostic value and enables better outcomes.

Conclusions

Noninvasive FDG-PET/CT enables the assessment of thrombus age and inflammation in experimental and clinical DVT, and enables the specific detection of recurrent murine DVT. Thrombus neutrophils are a major cellular basis of FDG signal in early murine DVT. Elevated FDG-PET signal indicates a recently formed, neutrophil-rich thrombus and thereby offers an imaging strategy to accurately diagnose recurrent same-site DVT.

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Disclosures

None.

References


we found that 18F-fluorodeoxyglucose (FDG)-positron emission tomography (PET)/computed tomography imaging allows the diagnosis and management of recurrent DVT in patients. Ultimately, prospective clinical studies are needed to evaluate if FDG-PET can improve the diagnosis and management of recurrent DVT in patients.
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SUPPLEMENTAL MATERIAL

Supplemental Methods

Ex vivo gamma counting and histopathology
After sacrifice, mice were perfused with 0.9% saline via the left ventricle. Radioactivity of excised jugular veins was measured by a gamma radiation counter (Wizard, PerkinElmer). In a subset of resected DVT (day 2 timepoint, n=5), the vein wall and thrombus were gently separated followed by gamma radioactivity measurements. Next, jugular veins were fixed overnight with paraformaldehyde (PFA) and embedded in optimal cutting temperature compound (Sakura Finetek, Torrance, CA). Serial 6-µm cryostat sections were obtained for H&E, Carstairs’ fibrin staining, Masson trichrome, and immunohistochemistry. Immunohistochemical detection of thrombus neutrophils (NIMP-R14, Santa Cruz Biotechnology, TX), macrophages (CD68, AbD Serotec, Oxford, UK), iNOS (Abcam, MA), and glucose transporter protein type 1 (GLUT-1, Santa Cruz) was performed. The number of thrombus neutrophils and macrophages per 5 high power fields (HPF; 1000x) was quantified as previously reported. As a positive controls for GLUT-1 and iNOS expression, we stained macrophage-rich atheroma sections from the aortic sinus of cholesterol-fed ApoE knockout mice.

Protein extraction and immunoblotting
Resected tissues were homogenized in 100-200 µl of RIPA buffer (Sigma-Aldrich) supplemented with protease inhibitor. Extracted proteins were separated by SDS PAGE
and transferred to PVDF membranes. Anti-matrix metalloproteinase-9 (MMP-9) antibody (Abcam) and anti-beta actin antibody (Sigma) were used.

**Clinical FDG-PET study.** The Partners Institutional Review Board approved access to patient records and analysis of patient data (Protocol #: 2011-P-000521). Participants for the clinical imaging sub-study were consecutively identified from a database of patients who had undergone 18-fluorine-fluorodeoxyglucose-positron emission tomography (¹⁸F-FDG-PET) and computed tomography (CT) imaging for oncological evaluation at the Massachusetts General Hospital. We used the clinical research database to generate an initial cohort of patients (n=437) with the following inclusion criteria: age >18, diagnosis of DVT by billing code, and receiving an ¹⁸F-FDG-PET and CT scan between 2004 through 2011 at Massachusetts General Hospital. Exclusion criteria included: deep vein thrombosis (DVT) outside the area of PET imaging, a DVT outside of the iliac-femoral veins, or a confounding reason for inflammation around the site of DVT (i.e. recent catheterization, vasculitis, nearby infection or tumor). This cohort was then restricted to patients with DVT occurring within 6 months after PET, leaving a total of 20 subjects. In one of these patients however an appropriate matched control could not be identified; therefore 19 patients, and 19 matched controls were included in the final analysis. Age of the DVT was determined as accurately as possible by clinical (n=17) and imaging evidence (n=2). Control subjects (n=19) without DVT who had undergone FDG imaging for clinical purposes were consecutively identified and matched 1:1 by gender, age (±10 years), active cancer and chemotherapy treatment within six months of imaging, and steroid immunosuppressive therapy at the time of
PET imaging, if present (Supplemental Table). Additionally, individuals with DVT were grouped according to the clinical age of their DVTs at time of imaging (divided into tertiles). Thereafter, the group-mean FDG signal was compared to the age of the DVT using linear regression analysis.

**PET/CT protocol.** Whole body FDG-PET imaging was performed per clinical protocol using a Biograph 64 Scanner (Siemens, Forcheim Germany) or similar system. FDG was administered ~370 MBq (~10 mCi) intravenously after an overnight fast. PET images were acquired in 3D mode ~60 minutes later. Patients were imaged in the supine position and images were obtained over 15-20 minutes. A low-dose, non-gated, non-contrast enhanced CT (120 keV, 50 mAs) was employed for attenuation correction prior to the PET scan. **FDG-PET/CT image analysis.** PET-CT images were analyzed by an investigator blinded to the patients’ clinical information. $^{18}$F-FDG uptake was measured within the right and left legs of both control and DVT subjects to assess uptake from the common femoral vein through the external iliac vein. An individual region-of-interest (ROI) was placed around the area just superior to the popliteal vein and extended to the inferior of the iliac bifurcation in order to obtain a maximum standardized uptake value (SUV$_{\text{max}}$). The average venous blood uptake of the right atrium was used to derive a target-to-background ratio (TBR) from the SUV$_{\text{max}}$. Patients were then analyzed to assess the time-dependent changes in FDG uptake in DVT and corresponding vein of matched control patients without a DVT.

**Statistics**
For animal data, results are expressed as median [25%-75% quartiles]. Statistical comparisons between two groups were evaluated by the Mann-Whitney U test, and by the Kruskal-Wallis test for multiple groups followed by the Dunn’s post-test. For comparison between two groups within the same animal, the Wilcoxon matched-pairs signed-rank test was used. Continuous variables at multiple timepoints were compared by the Friedman test. FDG uptake measurements were correlated with histological findings by use of the two-tailed Spearman method. Statistical comparisons were performed with GraphPad Prism (La Jolla, CA). Mice that did not develop jugular DVT (histologically negative, n=3) were excluded from analyses. For clinical data, values are expressed as mean±SEM. Wilcoxon signed ranks test was used for single comparisons, and after confirming normality of distribution, linear regression analysis was used to determine the association between FDG uptake and DVT age (SPSS 22, IBM, Chicago, IL). In the multivariable models, FDG uptake within the DVT was entered as the dependent variable, while DVT age was entered as an independent variable. Subsequently, clinical factors were added to the model as additional independent variables to assess their potential impact on the relationship between DVT age and FDG uptake. In order to limit the number of variables entered into the regression model at one time (given the modest sample size), the clinical variables were added to the multivariable model in three separate groupings: (1) demographic factors (patient age and gender); (2) factors that might impact systemic inflammation (immunosuppressive steroid use, history of recent infection, or statin use); and (3) oncologic history (history of cancer, cancer subtype, and history of chemotherapy). A value of $P<0.05$ was considered statistically significant.
### SUPPLEMENTAL TABLE

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**Supplemental Table.** Clinical characteristics of patients in the FDG-PET/CT study. Values are presented as mean±SD. BMI=body mass index; DVT= deep venous thrombosis.
Supplemental figure S1. Schematic experimental design. (A) Mice underwent FDG-PET imaging at various timepoints, followed by ex vivo analyses and histology. (B) Serial FDG-PET imaging at day 2, 7, and 14 was performed in a subset of three mice. Mice were sacrificed after the final PET imaging at day 14. (C) To assess the role of neutrophils in FDG signal, antibody-based neutrophil depletion was performed in subset of four mice before DVT formation and imaging. (D) For the recurrent DVT model (n=6 mice), we removed the suture of the ligation at day 2 to spur recanalization of the thrombosed right jugular vein, followed by re-ligation 12 days later to induce recurrent same-site stasis-induced DVT. We also performed complete ligation of the left contralateral jugular vein at day 2 to enhance recanalization of the initial right jugular DVT, and to compare a fresh recurrent DVT and an older DVT in the same mouse.
Supplemental figure S2. The time-dependent decrease in matrix metalloproteinase-9 (MMP-9) expression in DVT parallels the time-dependent decrease in the in vivo DVT FDG signal. (A and B) Immunoblotting of MMP-9 demonstrates time-dependent decrease. (C) DVTs from neutropenic mice shows decreased MMP-9 expression.
Supplemental figure S3. Little glucose transporter protein type 1 (GLUT-1) and iNOS expression is present in macrophages within day 14 DVT (top row, asterisk). Representative images of immunostaining (CD68-macrophage, iNOS-M1 macrophage marker, and GLUT-1) of day 14 DVT (top row), as well as of an aortic plaque on an ApoE knock-out mice showing higher expression of GLUT-1 and iNOS (bottom row, arrowhead). Asterisk=DVT. Scale bar, 100 µm.

SUPPLEMENTAL REFERENCE