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FDG-PET/CT Enables the Detection of Recurrent Same-Site Deep Vein Thrombosis by Illuminating Recently Formed, Neutrophil-Rich Thrombus

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Abstract

Background—Accurate detection of recurrent same-site deep vein thrombosis (DVT) is a challenging clinical problem. As DVT formation and resolution are associated with a preponderance of inflammatory cells, we investigated whether noninvasive ¹⁸F-fluorodeoxyglucose (FDG)-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/CT was performed at DVT timepoints of day 2, 4, 7, 14, or 2+16 (same-site recurrent DVT at day 2 overlying a primary DVT at day 16). Antibody-based neutrophil depletion was performed in a subset of mice prior to 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 compared to controls (p=0.03). Recurrent DVT demonstrated significantly higher FDG uptake than organized

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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.

Keywords

DVT; fluorodeoxyglucose; inflammation; recurrent DVT; PET; neutrophil

Introduction

Deep vein thrombosis (DVT) and subsequent pulmonary embolism is a major cause of cardiovascular death.^{1, 2} The incidence of DVT in industrialized countries is 1–3 individuals per 1,000 per year.^{1, 3} Furthermore, the risk of recurrent DVT is up to 10% *per year* in patients with idiopathic or unprovoked DVT.⁴ In addition, after DVT treatment, patients may experience recurrent leg pain due to a recurrent DVT, the post-thrombotic syndrome, or other etiologies. Failure to diagnose a recurrent DVT places the patient at risk of fatal pulmonary embolism.⁵ Therefore the accurate diagnosis of a recurrent DVT is critical to preserve health, as well as to justify prolonged, potentially life-long, anticoagulation and its attendant bleeding risks. Unfortunately, the diagnosis of a recurrent DVT often poses a significant diagnostic challenge for anatomical imaging methods such as duplex ultrasonography, computed tomography (CT) venography, or magnetic resonance venography, as a prior residual thrombus confounds the diagnosis of acute superimposed on remote thrombus.^{5, 6} Thus accurate approaches to identify recurrent DVT are urgently needed.

DVT formation and resolution are time-dependent inflammatory processes that involve neutrophils and macrophages.^{7, 8} Since 18-fluorine-fluorodeoxyglucose (FDG) uptake is upregulated in lesions containing pro-inflammatory myeloid cells such as neutrophils^{9–11} and macrophages,^{12, 13} we hypothesized that FDG-positron emission tomography (PET)-CT could allow noninvasive imaging of DVT-induced inflammation, and allow the identification of recurrent DVT even in sites with pre-existing 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 jugular vein

Animal studies were approved by the MGH Subcommittee on Research Animal Care. Male C57BL/6 mice (14–20 weeks) were anesthetized 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.¹⁴ As a sham control, the left jugular vein was surgically exposed and tied loosely without constriction (**Fig. 1A**). At various timepoints after ligation from day 2 up to day 16, PET/CT imaging was performed, followed by sacrifice and ex vivo analyses (n=4-6 at each timepoint). A subset of mice (n=3) underwent three-timepoint serial PET/CT imaging and were sacrificed after the final imaging timepoint (**Supplemental Fig. S1**). 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 re-ligation 12 days later to induce recurrent same-site stasis-induced DVT (**Supplemental Fig. S1**). 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 15-25 $\mu\text{Ci/g}$ of ^{18}F -FDG (PETNET Solutions, Woburn, MA). One hour later, microPET/CT imaging was performed using a small animal scanner (Inveon, Siemens Medical Solutions, Inc., Malvern, PA). CT imaging preceded PET imaging, with acquisition of 360 cone beam projections using a source power and current of 80 keV and 500 A, respectively. Mice were injected with an iodinated contrast agent (Isovue-370) at 20 $\mu\text{L/min}$ during the CT scan. Projections were reconstructed into 3-dimensional volumes containing $512 \times 512 \times 768$ voxels with the dimension of $0.11 \times 0.11 \times 0.11$ mm (Amira, San Diego, CA). The PET image voxel size was $0.797 \times 0.861 \times 0.861$ mm. Data were calculated as mean and max standardized uptake values (SUVs) and TBR (target-to-background ratio; DVT/sham).

Induction of neutropenia.—A subset of mice (n=4) was treated with anti-neutrophil antibody (Accurate Chemical, NY) to induce neutropenia.^{15, 16} The antibody was initially injected (500 $\mu\text{g/mouse}$, i.p.) one day prior to surgery. Additional doses of the antibody were i.v. administered (250 $\mu\text{g/mouse}$) on postoperative days 1 and 2 (**Supplemental Fig. S1C**).

Ex vivo gamma counting and histopathology

See Supplement for full details. 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. The number of thrombus neutrophils and macrophages per 5 high power fields (HPF; 1000x) was quantified as previously reported.¹⁷

Protein extraction and immunoblotting

See supplement.

Clinical FDG-PET study

See 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 ^{18}F -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 iliofemoral 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 (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_{max}). The average venous blood uptake of the right atrium was used to derive a TBR.¹⁸ 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 Supplement for full details. 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 $\pm$ SEM. The Wilcoxon signed-rank 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). 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 due to high FDG background in the kidneys and spine, as well as the surrounding bowel, an organ with high glucose utilization.¹⁹ 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 DVT were readily detected as obstructions on CT venography, in contrast to sham-operated contralateral jugular veins (**Fig. 1A-B**). Histological analyses confirmed fibrin, red blood cells, and white blood cells, similar to the ligation IVC stasis model (**Fig. 1C-E**).^{14, 17, 20}

Noninvasive imaging of DVT inflammation by FDG-PET/CT

In vivo ^{18}F -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 (**Fig. 2A, FDG-PET**). Thrombosed jugular veins induced a filling defect on CT venography (**Fig. 2B, CT**) that colocalized well with FDG-PET signals (**Fig. 2C, PET/CT**).

^{18}F -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 ^{18}F -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, $p < 0.001$, **Fig. 3A**). The DVT SUV_{max} and TBR values were highest at day 2 and decreased thereafter (SUV_{max} = 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, $p = 0.002$, **Fig. 3B-C**). Gamma radiation counts of resected DVT demonstrated similar findings, with a time-dependent decrease in radioactivity (%IDGT = 7.81 [6.47-19.2]% 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, vs. 0.735 [0.458-1.75]% (pooled sham), $p < 0.0001$, **Fig. 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 ($p = 0.03$, **Fig. 3E-F**).

Neutrophil appearance associates with FDG uptake in DVT

We compared ^{18}F -FDG uptake measurements and histological profiles at the study timepoints of 2, 4, 7, and 14 days after induction of stasis DVT. Neutrophils infiltrated DVT abundantly at the earlier timepoints of day 2 and 4, with fewer neutrophils observed at day 7, and minimal neutrophils at day 14 (**Fig. 4**). Thrombus macrophages were evident at day 7 and resided in the outer DVT edge (**Fig. 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; $r = 0.62$, $p < 0.001$, **Fig. 5A**). The macrophage FDG relationships were analyzed in day 7 and day 14 DVT, as 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; $r = 0.26$, $p = 0.23$, **Fig. 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; $r = 0.30$, $p = 0.37$, **Fig. 5C**).

To further examine the relationship of FDG-PET and thrombus inflammation, we assessed the protein expression of matrix metalloproteinase-9 (MMP-9), a key inflammatory mediator of DVT.⁸ Immunoblot analyses showed a time-dependent decrease of MMP-9 expression in DVT (**Supplemental Fig. S2A-B**, $p = 0.01$), 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 $66.7 \pm 5.9\%$ of the radioactivity localized in thrombus. We further analyzed the distribution of neutrophils using immuno-histological images. We observed $86.4 \pm 1.4\%$ of 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.

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 prior to DVT induction. Pre-treatment with an anti-neutrophil antibody decreased circulating neutrophils in day 2 DVT mice ($0.6 [0.2-0.9] \times 10^3/\mu\text{L}$ vs. control day 2 DVT mice, $1.7 [1.2-2.1] \times 10^3/\mu\text{L}$, $p=0.03$). On day 2, imaging demonstrated a significant decrease in FDG-DVT signal in neutropenic mice, compared with normal mice (SUV = $1.15 [1.06-1.51]$ vs. $1.98 [1.87-2.70]$, $p=0.03$, **Fig. 6**). Histological assessment confirmed diminished neutrophil accumulation in DVT (neutropenia $21.0 [14.0-59.0]$ vs. control $102 [86.0-126]$, # neutrophils per 5 high-power-fields, $p<0.001$). Neutropenic mice also showed decreased expression levels of MMP-9 in DVT in parallel to FDG signal (**Supplemental Fig. S2C**).

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 due to 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 de-ligated (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 de-ligated vein was then re-ligated at same location (**Fig. 7A**). This procedure successfully generated a second new venous thrombus at day 2 directly overlying the 16 days 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 (**Fig. 7B-D**). The vein wall also exhibited DVT-induced scarring (increased thickness and collagen-rich), which is observed in patients with post-thrombotic syndrome (PTS).²¹ A number of neutrophils were present in day 2 recurrent DVT, while macrophages were abundant in day 16 DVT, similar to day 14 DVT (**Fig. 7E-F**).

FDG-PET/CT enables the identification of recurrent, same site DVT

As 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 Fig. 7G-I, 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] vs. 0.962 [0.892-1.07], SUV_{max}; 2.01 [1.63-2.17] vs. 1.06 [0.973-1.16], $p=0.03$ respectively, **Fig. 7J-K**). Ex vivo gamma radiation measurement also showed a significant increase in the activity of recurrent DVT (day 2+16) compared to older DVT at day 14 (%IDGT = 20.46 [16.4-26.3]% vs. 4.60 [2.46-7.75]%, $p=0.02$, **Fig 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 (**Supplemental Table**). We observed a significantly increased signal within recently formed DVT (**Fig. 8A-B**). Across the entire population, the FDG signal in DVT was significantly greater than in the matched vein of controls (SUV_{max} 1.87±0.15 vs. 1.32±0.10, $P=0.02$; TBR 1.62±0.19 vs. 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 SUV_{max} and TBR of DVT diminished over time ($p=0.002$ for SUV_{max}, $p=0.004$ for TBR, **Fig. 8C-D**). The relationship between DVT age (by tertiles) and DVT FDG uptake (either as SUV_{max} or TBR) remained significant after multivariable adjustments for: demographic factors, (TBR: $\beta=-0.209$, $p=0.007$; SUV_{max}: $\beta=-0.233$, $p=0.003$); factors that could impact systemic inflammation (TBR: $\beta=-0.228$, $p=0.002$; SUV_{max}: $\beta=-0.237$, $p=0.003$); and oncologic history (TBR: $\beta=-0.225$, $p=0.003$; SUV_{max}: $\beta=-0.245$, $p=0.001$).

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 utilizing 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 DVT⁴ and is a highly morbid condition, increasing the risk of the post-thrombotic 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 IVC filter placement 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 endpoint in observational and therapeutic clinical venous thromboembolism (VTE) trials,² there is no gold standard for its diagnosis, including

ultrasound, MRI, CT, or venography.^{5, 6, 24} 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 SPECT imaging.^{29, 30} However, no established imaging technique is routinely utilized in the clinic.⁵ The current results 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, while other clinical reports have demonstrated that FDG-PET can identify DVT (but not specifically recurrent DVT),^{32, 33} our experimental results extend these prior studies, as 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.

As 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 < 10 days old.³⁶ As 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^{12, 37} and neutrophils.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, as M2-macrophages do not exhibit up-regulated glucose transporter protein type 1 (GLUT-1) expression needed to concentrate FDG.^{12, 39} Histological analyses supported this hypothesis, as macrophages within day 14 DVT in fact displayed little GLUT-1 expression and little iNOS expression, an M1 marker (**Supplemental Fig. S3**). This is in distinction to chronic inflammatory conditions such as in atherosclerosis, where macrophages account for the FDG signal,^{13, 37, 40-43} and demonstrate

an M1-polarized (pro-inflammatory) 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 As there was a mild FDG signal in day 14 DVT without neutrophils, other non-macrophage 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. As 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 wound healing region around the DVT and likely in the venous wall. While experiments demonstrate that the majority of FDG signal arises from the thrombus, the vein wall also contributes modestly to the FDG signal. As 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 oncologic 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, while it is known that ¹⁸F-FDG uptake is upregulated in lesions containing pro-inflammatory myeloid cells such as neutrophils⁹⁻¹¹ and macrophages,^{12, 13} histological analysis of clinical DVTs was not possible to perform 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 compared to the matched vein of control patients. By analyzing greater numbers of DVT patients and with clot ages up to 21 weeks old, 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 employed 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 post-thrombotic syndrome.^{46, 47} 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 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Induction of stasis-induced DVT model in the murine jugular vein. (A) Surgical ligation of the right jugular vein was performed to induce deep vein thrombosis (DVT). As a sham control, the left jugular vein was surgically exposed and loosely tied without constriction. (B) Contrast-enhanced computed tomography (CT) venography shows a filling defect only in the right jugular vein with DVT (dark signal, yellow arrow). Surgery-induced air artifact was observed in front of ligated jugular vein (asterisk). (C) Resected red-white thrombus in the ligated jugular vein. (D) Hematoxylin-eosin stain. (E) Carstairs' stain (red = fibrin, yellow = erythrocytes, blue = collagen). Representative DVT images at day 0 (A) and day 4 (B-E). Scale bars, 500 μ m.

Figure 2.

FDG-PET enhances acute DVT in mice. Representative images of (A) FDG-PET (B) contrast-enhanced CT venography, and (C) fused PET/CT at various timepoints. DVT (yellow arrow) induced filling defects in contrast CT venography not seen in the contralateral sham-operated vein (white arrow). Surgery-induced air artifact (dark signal) is observed anterior to each vein on CT (white asterisk). FDG-PET DVT signal was elevated in acute DVT, and diminished over time. Mild surgery-induced inflammation at wound healing region was observed in the sham surgery left jugular vein region at day 2.

Figure 3.

Time-course quantitative changes of FDG signal in DVT. (A) Standard uptake value (SUV), (B) SUV_{max}, (C) target-to-background ratio (TBR), and (D) %injected dose per gram of tissue (%IDGT) 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.

Figure 4.

Recruitment of inflammatory cells into DVT. (A) Representative immunostaining of neutrophil and macrophage from various DVT timepoints. Neutrophils are abundant and predominate in early day 2-4 DVT. Thrombus macrophages were evident from day 7 and resided at the outer DVT edge. (B) The number of neutrophils (black) and macrophages (white) per 5 HPF (high power field) were shown. (* $p < 0.0001$). Scale bar, 500 μm . n.s.=not significant.

Figure 5.

FDG signal associations with thrombus neutrophils and macrophages. (A) The number of thrombus neutrophils correlated with the FDG uptake in DVT (SUV; $r=0.41$, $p=0.004$, TBR; $r=0.62$, $p<0.001$). (B) Thrombus macrophages pooled from day 7 and day 14 did not significantly correlate with the FDG-DVT signal (SUV; $r=-0.15$, $p=0.47$, TBR; $r=0.26$, $p=0.23$). (C) Sub-analysis of macrophages at day 14 also did not significantly correlate with FDG-DVT signals (SUV; $r=0.04$, $p=0.91$, TBR; $r=0.30$, $p=0.37$). MAC= macrophage.

Figure 6.

Experimental neutropenia reduces FDG signal within DVT. (A) Representative images of contrast-enhanced CT and fused PET/CT from neutropenic mice (left) and uninjected control (right) are shown. DVT (yellow arrow) and sham-operated jugular vein (white arrow) are indicated by arrows. (B) SUV, SUV_{max}, and TBR of FDG signal of DVT in neutropenic mice are significantly lower than control mice (*p<0.05). N=4 per group. Median SUV and SUV_{max} value of sham-operated contralateral jugular veins are shown as respective dotted lines.

Figure 7.

Establishment of a novel recurrent DVT model, and specific detection of recurrent DVT by FDG-PET/CT. (A) Two days after the initial ligation, the suture was de-ligated and removed. At day 14, re-ligation was performed at previously ligated site to induce a recurrent DVT. Histological images of resected recurrent DVT (day 2 recurrent DVT (outlined with black dotted line) overlying the day 16 DVT (outlined with blue dotted line)) are shown. (B) Hematoxylin-eosin, (C) Carstairs' staining, and (D) Masson Trichrome staining shows red blood cell-rich zones in recurrent DVT (red in H&E and yellow in Carstairs') and collagen-rich zones in older DVT (pink in H&E and blue in Carstairs' and Masson). (E and F) Immunostaining of neutrophils (E) and macrophages (F) shows the newly formed recurrent DVT is neutrophil-rich, while the older primary DVT shows macrophage predominance. Representative (G) PET, (H) CT, and (I) PET/CT images are shown. FDG enhanced recurrent DVT substantially more than older DVT (G and I). (J-L) FDG signal was significantly higher in recurrent DVT (day 2 overlying the day 16 DVT) than in older day 14 DVT in the contralateral vein. N=6 per group. Scale bar, 500 μ m.

Figure 8.

¹⁸F-FDG DVT signal is elevated in patients and diminishes over time. Representative PET/CT images from (A) a patient with DVT and (B) a matched control patient without DVT. Elevated FDG signal was observed in the thrombosed femoral vein (A, yellow arrow). (C) The SUV_{max} and (D) TBR of the DVT exhibited a time-dependent decrease ($p=0.002$ for SUV_{max}, $p=0.004$ for TBR, $n=6-7$ per DVT group). FDG uptake in the DVT vein was shown for individuals grouped according to age of the DVT, divided into tertiles. Values from the matched vein from control patients are shown in the No DVT group ($n=19$ patients).