Published in final edited form as:

J Neurooncol. 2012 April ; 107(2): 365–372. doi:10.1007/s11060-011-0750-y.

Increased tryptophan transport in epileptogenic dysembryoplastic neuroepithelial tumors

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Abstract

Dysembryoplastic neuroepithelial tumors (DNTs) are typically hypometabolic but can show increased amino acid uptake on positron emission tomography (PET). To better understand mechanisms of amino acid accumulation in epileptogenic DNTs, we combined quantitative α - ^{11}C methyl-L-tryptophan (AMT) PET with tumor immunohistochemistry. Standardized uptake values (SUVs) of AMT and glucose were measured in 11 children with temporal lobe DNT. Additional quantification for AMT transport and metabolism was performed in 9 DNTs. Tumor specimens were immunostained for the L-type amino acid transporter 1 (LAT1) and indoleamine 2,3-dioxygenase (IDO), a key enzyme of the immunomodulatory kynurenine pathway. All 11 tumors showed glucose hypometabolism, while mean AMT SUVs were higher than normal cortex in eight DNTs. Further quantification showed increased AMT transport in seven and high AMT metabolic rates in three DNTs. Two patients showing extratumoral cortical increases of AMT SUV had persistent seizures despite complete tumor resection. Resected DNTs showed moderate to strong LAT1 and mild to moderate IDO immunoreactivity, with the strongest expression in tumor vessels. These results indicate that accumulation of tryptophan in DNTs is driven by high amino acid transport, mediated by LAT1, which can provide the substrate for tumoral tryptophan metabolism through the kynurenine pathway, that can produce epileptogenic metabolites. Increased AMT uptake can extend to extratumoral cortex, and presence of such cortical regions may increase the likelihood of recurrent seizures following surgical excision of DNTs.

Keywords

Dysembryoplastic neuroepithelial tumor; Epilepsy; Positron emission tomography; α - ^{11}C Methyl-L-tryptophan; L-Type amino acid transporter 1; Indoleamine 2,3-dioxygenase

Introduction

Dysembryoplastic neuroepithelial tumors (DNTs) are low-grade glioneuronal tumors most often located in the temporal lobe [1]. DNTs are typically diagnosed in children and young adults following new-onset partial seizures [1, 2]. Intractable seizures often warrant resective surgery [3, 4]. Despite some distinctive MRI features, DNTs are often difficult to differentiate from non-neoplastic lesions or infiltrating gliomas. Functional imaging with positron emission tomography (PET) can evaluate tumor grade and detect potentially epileptogenic cortex. DNTs are typically hypometabolic on 2-deoxy-2- ^{18}F fluoro-D-glucose (FDG) PET [5–7]. ^{11}C Methionine PET studies have shown low tracer uptake in most DNTs [7–9]. In contrast, a recent PET study reported high uptake of ^{18}F fluoroethyl-L-tyrosine (FET) in four of six DNTs [10]. The authors hypothesized that increased FET uptake was mediated by the L-type amino acid transporter (LAT1).

α - ^{11}C methyl-L-tryptophan (AMT) is an amino acid PET radiotracer, which is not a substrate for protein synthesis but is suitable to estimate local brain serotonin synthesis and the immunomodulatory kynurenine pathway [11–16]. High interictal AMT uptake on PET can identify epileptogenic tubers and malformations of cortical development [17–21]. Increased tryptophan metabolism via the kynurenine pathway, producing neurotoxic and epileptogenic products (such as quinolinic acid [15]), may play a role in the epileptogenicity of these lesions. Recent studies have also implicated abnormal tryptophan metabolism via the kynurenine pathway in various forms of generalized and partial epilepsy [22, 23]. In recent PET studies, markedly increased uptake of AMT was reported in most human brain tumors (including those not associated with seizures) [11–13]. Most of these tumors showed expression of indoleamine 2,3-dioxygenase (IDO), the initial and rate-limiting enzyme of the kynurenine pathway [12]. Upregulation of IDO has been implicated in glioma immune

resistance [24]. The substrate of this enzyme, L-tryptophan, is mostly extracted from the blood via LAT1. The degree of LAT1 expression correlated with neutral amino acid (L-methyl-¹¹C-methionine) uptake in human gliomas [25]. Since DNTs showed lower tryptophan metabolic rates than astrocytomas (but similar AMT kinetics to oligodendrogliomas) [13], it could be hypothesized that increased tryptophan transport could be the driving force of AMT uptake seen in DNTs.

The main goal of the present study was to evaluate mechanisms of tryptophan accumulation in epileptogenic DNTs. Detailed kinetic analysis of dynamic AMT PET images was combined with assessment of LAT1 and IDO expression patterns using immunohistochemistry in surgical DNT specimens. In addition, we evaluated if degree of AMT uptake is related to glucose metabolic rates in DNTs. Finally, we also studied whether AMT PET can detect abnormal cortex beyond the MRI-defined tumor mass, and if such cortical areas may be relevant for seizure control following surgical resection of the neoplasm.

Materials and methods

Study population

Eleven children (age range: 2.5–17.5 years; median: 10.9 years) with epilepsy and a histopathological diagnosis of WHO grade I DNT in the temporal lobe were included (Table 1). All patients underwent MRI as well as FDG PET as part of their clinical investigation, whereas AMT PET scanning prior to tumor resection was part of a research protocol. Written informed consent by parents and written assent from children over the age of 13 years was obtained. In addition, resected tissues were examined for expression of IDO and LAT1 by immunohistochemistry. Duration between the first seizure and AMT PET ranged between 1 month and 9.5 years (median: 7 months).

MRI acquisition protocol

Diagnostic MRI studies were performed on a GE 1.5T Signa 5.6 Unit (GE Medical Systems, Milwaukee, WI). The MRI protocol always included T1-weighted volumetric spoiled gradient echo (SPGR) (in 10 patients), an axial T1-weighted pre- and post-gadolinium, axial and coronal T2-weighted, and coronal fluid attenuated inversion recovery (FLAIR) sequences.

FDG and AMT PET scanning protocol

All 11 AMT PET scans and nine of the FDG PET scans were acquired using an EXACT/HR PET scanner (CTI/ Siemens, Knoxville, TN), which provides 47 contiguous transaxial images (slice thickness: 3.125 mm). Two FDG PET scans were acquired on a GE Discovery STE PET/CT scanner (GE Medical Systems, Milwaukee, WI) with an isotropic image resolution of 6.0 ± 0.5 mm at full-width at half-maximum (FWHM). For FDG PET on the CTI/Siemens scanner the reconstructed image resolution was 5.5 ± 0.35 mm at FWHM in-plane. Scalp EEG was monitored in all patients during the FDG uptake period. Initially, 0.143 mCi/kg of FDG was injected intravenously as a slow bolus followed by a 30-min uptake period. 40-min after injection, a static 20-min emission scan was acquired parallel to the canthomeatal plane. All scans were acquired in the interictal state.

The AMT PET studies were performed with a reconstructed image in-plane resolution of 7.5 ± 0.4 mm at FWHM. The procedure for AMT PET scanning has been described previously [11]. In brief, after 6 h of fasting, a venous line was established for injection of AMT (0.1 mCi/kg) as a slow bolus over 2 min. After injection of AMT, a 20 min dynamic PET scan of the heart was performed (sequence: 12×10 , 3×60 , and 3×300 s) to obtain the left ventricular (LV) input function. Continuation of the blood input function beyond this initial

20 min was achieved using venous blood samples. At 25 min after tracer injection, a dynamic emission scan of the brain (7×5 min) was acquired. EEG was not monitored during the AMT PET studies, as we have previously demonstrated that epileptiform activity did not affect AMT kinetic variables in epileptogenic brain tumors [11]. The interval between the AMT and FDG PET scans ranged from 1 to 139 days (mean: 22 days).

Image analysis

The interval between MRI and AMT PET ranged from 0 to 103 days (mean: 36 days). FDG and AMT PET images were co-registered to each patient's SPGR (or FLAIR) MR images using VINCI 2.50 [26]. Regions of interest (ROIs) encompassing the entire tumor mass were defined using the software ROI Editor 1.4.1 (www.mristudio.org) on the MR images and superimposed on the co-registered FDG and AMT PET images including the dynamic AMT image sequences in cases where blood input data were available (nine subjects). In addition, contralateral temporal cortical ROIs were drawn and superimposed onto co-registered PET images as internal controls.

Standardized uptake values (SUVs) for both FDG and AMT as well as corresponding tumor/contralateral cortex SUV ratios were calculated. Kinetic analysis of AMT uptake was carried out using the Patlak graphical analysis as described previously [11, 27]. This approach utilizes the dynamic brain sequence and the blood input function to provide two kinetic parameters calculated from the Patlak plot: the slope parameter that corresponds to the K-complex reflects the unidirectional uptake of the tracer into tissue [11, 15]. The y axis-intercept of the plot provides the volume of distribution (VD), which characterizes the transport rate of AMT into the analyzed tissue.

Immunohistochemical analysis

Adequate tissue samples were available for immunohistochemical studies from eight DNTs. Polyclonal rabbit antibodies were used on formalin-fixed, paraffin-embedded sections (5 μ m) for detection of IDO (rabbit anti-IDO, AB9898, Millipore Corporation, Billerica, MA) and LAT1 (SLC7A5 antibody, ab85226, Abcam, Cambridge, MA). Secondary detection used the ABC Elite system (Vector Laboratories, Burlingame, CA) and DAB Enhanced Liquid Substrate System (D3939, Sigma-Aldrich, St. Louis, MO). Counterstaining of nuclei was performed with hematoxylin. Imaging was performed using an Olympus BX51 microscope (Olympus Corporation, Center Valley, PA).

For semiquantitative analysis, immunohistochemical staining intensity of DNT cells (including all extra-vascular cells, mostly neurons and oligodendroglioma-like cells) as well as tumor blood vessels was rated separately from 0 to 3 (0 = no staining; 1 = mild staining; 2 = moderate staining; 3 = strong staining) in at least three representative 20 $\times$ magnification fields of view of all DNT samples by one of the investigators who was blinded to the identity of the specimens. The intrarater reliability (based on intraclass correlation) of the scoring was determined to be between 0.83 and 0.95 by repeated analysis of random fields of view.

Subsequently, a weighted average staining intensity score for both DNT cells and blood vessels was calculated, as follows:

$(3 \times \text{No. of cells/vessels with score 3} + 2 \times \text{No. of cells/vessels with score 2} + 1 \times \text{No. of cells/vessels with score 1}) / \text{No. of all analyzed cells/vessels}.$

Study design and statistical analysis

Spearman's product moment correlation was applied for correlations between tumor volumes, FDG and AMT SUV values and tumor/cortex SUV ratios, as well as between imaging and clinical (age at scan, age at seizure onset) variables. Immunohistochemical staining scores were compared between tumor blood vessels and DNT cells as well as between LAT1 and IDO staining scores using a Wilcoxon signed rank test. Statistical analysis was performed using PASW Statistics 18 (SPSS Inc., Chicago, IL, USA). A *P* value less than 0.05 was considered to be significant.

Results

Imaging characteristics and AMT kinetics in DNTs

All 11 tumors showed glucose hypometabolism on FDG PET scans (SUV tumor/cortex ratio: 0.29–0.64). Tumor AMT SUV values were higher than cortical values in 8/11 cases (see example on Fig. 1), including all three contrast-enhancing DNTs. The three tumors with an average AMT SUV below cortical values could be identified visually on the summed AMT uptake images (30–55 min post-injection), because they showed higher uptake than surrounding white matter in the medial temporal region. AMT uptake was heterogeneous within the tumor in two of these cases and the maximal SUV values within the tumors exceeded cortical values. There was no correlation between tumor FDG and AMT SUV values or tumor/cortex SUV ratios ($P > 0.13$).

Kinetic analysis of dynamic AMT PET images showed markedly increased VD values, as compared to normal cortex, in 7/9 children (≥ 1.40 tumor/cortex VD ratios). The remaining two DNTs showed slightly decreased VD values. Small increases in K-complex tumor/cortex ratios (range: 1.05–1.08) were found in three patients (Table 1). None of the FDG and AMT PET variables showed a correlation with age or age at seizure onset ($P > 0.06$).

Epilepsy surgery and cortical AMT increases

Eight of the ten children with at least 1 year of follow-up (mean $\pm$ SD: 5.0 ± 2.9 years) had seizure-free surgical outcomes, while two (patients #3 and #9) had persistent complex partial seizures despite complete tumor resection. Only these latter two patients had increased uptake of AMT in cortical areas not involved in surgical resection: one in the ipsilateral fronto-temporo-parietal (AMT SUV ipsilateral/contralateral cortex ratio: 1.21; Fig. 2) and the other in the ipsilateral parietal cortex (AMT SUV ratio: 1.10). These extratumoral cortical regions with increased AMT uptake showed no structural abnormality on MRI, no glucose hypometabolism on FDG PET. Rare interictal spiking activity was seen in the peritumoral cortex on intraoperative electrocorticography, but no long-term intracranial EEG monitoring was done to capture seizures.

IDO and LAT1 immunohistochemistry in DNTs

All eight DNTs available for immunohistochemistry showed expression of both LAT1 and IDO, with immunoreactivity in both blood vessels and DNT cells (Table 1; see examples on Fig. 3). LAT1 staining was present in virtually all blood vessels, where it showed moderate or strong staining intensity. Staining was observed commonly in DNT neurons and occasionally in oligodendrogloma-like tumor cells (Fig. 4). Mean staining intensity scores for both IDO and LAT1 were higher in tumoral endothelial cells than in DNT cells (LAT1 scores: 2.8 vs. 1.1, respectively, $P = 0.012$; IDO scores: 2.4 vs. 1.4, respectively, $P = 0.012$). The intensity of LAT1 staining was significantly higher than IDO staining in tumor blood vessels ($P = 0.028$), while DNT cell staining for LAT1 and IDO showed similar intensities ($P = 0.48$).

Discussion

The present study demonstrates that increase in DNT tryptophan uptake on PET is mostly driven by high transport, although abnormal tryptophan metabolism by the kynurenine pathway may also contribute to AMT accumulation. Moreover, increased AMT uptake can extend beyond epileptogenic DNTs into cortical regions with normal appearance on MRI and FDG PET. Notably, only patients with such cortical increases had persistent seizures after complete tumor resection. Despite the limited numbers, this indicates that cortical areas with increased AMT uptake should be addressed during presurgical evaluation in order to maximize chances of seizure-free outcome.

Previous FDG and MET PET studies concluded that DNTs show low glucose and protein metabolic activity [5, 6, 9]. In contrast, uptake of FET, a PET tracer whose accumulation mostly reflects amino acid transport, was increased in 2/3 of DNTs in a recent small study [10]. The reason for this apparent discrepancy between MET versus FET (and also AMT) uptake of DNTs is unclear. Low MET uptake in most DNTs may be explained by the fact that microvascular density, tumoral blood flow and related passive diffusion play a greater role in tumoral uptake of MET as compared to some other amino acid radiotracers [28–31]. Still, since MET is a substrate of LAT1, it is also possible that relatively low MET uptake in DNTs reflects a limited retention of this radioligand (perhaps due to rapid metabolism via pathways other than protein synthesis) within the tumor tissue.

AMT is fundamentally different from other amino acid PET radiotracers in that it often shows accumulation in epileptic foci in the interictal state. In tuberous sclerosis, high AMT uptake identifies epileptogenic tubers with a very high specificity: tubers with at least 10% increase (as compared to normal cortical values), were all epileptogenic [19]. However, at least 1/3 of epileptogenic tubers and approximately half of epileptogenic cortical dysplasias show low AMT uptake [18–21]. Epileptogenic DNTs may show an even higher rate of increased AMT uptake: all but one of these tumors showed increased SUV in the tumor as a whole or at least in tumor subregions in our study. Kinetic PET analysis indicates that strong tumoral tryptophan transport is the major reason for tryptophan accumulation in DNTs. Immunostaining scores indicate a substantial presence of LAT1 in these tumors, with the bulk of this transport protein being seen in tumoral blood vessels. LAT1 is expressed in the blood–brain barrier [32–34]. In gliomas, upregulation of LAT1 in both endothelial and neoplastic cells can provide substrate for excessive protein synthesis associated with proliferation [35]. In contrast, high amino acid transport in indolent DNTs and similar LAT1 expression in balloon cells of tuberous sclerosis [36] is not likely to serve demands for high protein synthesis rates. Rather, increased tryptophan influx facilitated by LAT1 may provide the substrate for IDO, a key enzyme of the kynurenine pathway. Since all DNTs showed extensive, moderate to strong LAT1 expression in all vessels in our study, microvascular density may be an important factor affecting total tryptophan transport into the tumor parenchyma. Although we did not perform a formal correlation between staining scores and AMT kinetic variables due to the limited numbers, the role of vascular density in AMT transport may be supported by the fact that the two patients with the highest (patient 1, Fig. 3a) and lowest (patient 7) vascular density showed the highest and lowest AMT transport (VD) ratios, respectively, (Table 1). This potential relation between microvascular density and tryptophan transport deserves further studies in larger tumor samples.

IDO as well as other downstream enzymes have been found in cultured human malignant glioma cells [24] and low-grade brain tumors [12]. Tryptophan depletion associated with accumulation of toxic kynurenine metabolites can inhibit tumor infiltrating cytotoxic T cells, which can lead to immune resistance of tumors. On the other hand, tryptophan depletion may also curb tumor proliferative activity [37–39], which is consistent with the typical slow

growth pattern of DNTs, which can be harbored in the brain for many years without significant increase in size. In accordance with this, high IDO expression in endothelial cells of renal cell carcinomas was associated with lower tumor proliferative activity and longer survival [40]. Whether a similar effect exists in low-grade brain tumors, including DNTs, can be addressed in the future by measuring not only IDO but also various downstream metabolites of the kynurenine pathway in these tumors. An increase in quinolinic acid (a neurotoxic and epileptogenic kynurenine metabolite) in DNTs, similar to what was found in epileptogenic tubers [15], could also be related to the strong epileptogenicity of these tumors. Indeed, the kynurenine pathway can be activated by the release of proinflammatory cytokines, which have been implicated in the pathophysiology of epilepsy, especially in the context of cortical dysplasia and glioneuronal tumors [41–43]. Several cytokines, including IFN- γ , are stimulated by seizures and serve as inducers of IDO [44, 45], and they may represent a potential pharmacological target for treatment of epileptogenic lesions.

Another potentially important observation is that seizures persisted after tumor resection only in the two patients where increased AMT uptake was also seen in extratumoral cortical areas. Peritumoral infiltration is an unlikely explanation for these cortical increases, as grade I DNT-s are not known to show infiltrative properties beyond the actual tumor margin. It is more likely that these extratumoral regions represented epileptic cortex. This is supported by our prior studies demonstrating a very high specificity of increased AMT uptake for epileptic cortex [18, 46]. Intracranial EEG monitoring of such cortical regions may help further address this issue. Extratumoral areas of increased AMT uptake in our study could also indicate occult cortical dysplasias, a common DNT-associated pathology [4]. The ability of AMT PET to identify epileptogenic cortical dysplasia has been established [20, 21]. Less frequently, increased AMT uptake can also occur in non-lesional epileptic cortex [18, 20, 21], potentially indicating a chronic inflammatory process involving upregulation of the kynurenine pathway, although etiology of AMT accumulation in non-lesional epileptic cortex is not clarified. It has been also shown that multiple epileptogenic regions often exist in conjunction with distinct developmental malformations, including DNTs, and the likelihood of such multiple epileptic foci increases with longer duration of epilepsy [47]. Thus, although our sample mostly included patients with recent onset epilepsy, AMT PET could be particularly helpful in patients with a long history of DNT-associated epilepsy to evaluate potentially epileptic cortex outside the lesion and optimizing surgical resection and seizure outcome.

Acknowledgments

The study was supported by a grant from the National Institutes of Health (NIH, CA123451 to C.J.) and Start-up Funds (Wayne State University School of Medicine to S.M.). We thank Pulak Chakraborty, PhD, for AMT radiochemistry and Sam Kiouis, BS, for skilled technical assistance with immunohistochemistry. We are grateful to the entire staff at the PET Center, Children's Hospital of Michigan, who provided invaluable technical help in performing the PET scans.

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Fig. 1. Co-registered coronal slices of MRI (a), FDG PET (b), and AMT PET (c) images of a 10 year-old girl (patient #4) with a non-enhancing left inferior temporal DNT (*arrow*). FDG PET showed hypometabolism (FDG SUV tumor/cortex ratio: 0.45), while AMT PET demonstrated markedly increased tryptophan uptake (AMT SUV tumor/cortex ratio: 1.27) in the lesion

Fig. 2. Co-registered axial SPGR MRI (a), FDG PET (b), and AMT PET (c) images of a 6 year-old boy (patient #9) with a *left* medial temporal DNT (arrow). FDG PET demonstrated glucose hypometabolism of the tumor (FDG SUV tumor/contralateral cortex ratio: 0.29), and AMT PET showed no prominent tryptophan accumulation in the tumor volume (AMT SUV ratio: 0.99). However, an extensive area of ipsilateral cortex (mostly temporal, extending into parietal and occipital regions) showed a marked increase of AMT uptake (arrowheads) (SUV ipsilateral/contralateral cortex ratio: 1.21). Note that AMT accumulation seen in the bilateral occipital cortex and in the visualized portions of the basal ganglia is physiologic

Fig. 3.

Immunohistochemistry for expression of the LAT1 and IDO in two DNTs. **a** A highly vascular tumor (patient #1, with the highest AMT SUV and VD-ratio of all patients) with prominent LAT1 and moderate to strong IDO expression in tumor blood vessels (such as the ones indicated by *solid arrows*). DNT neuronal cells showed variable (most commonly mild) staining for both proteins. The *dashed arrows* indicate tumor neurons with mild to moderate LAT1 (*left*) and IDO (*right*) staining intensity. Oligodendrogloma-like tumor cells rarely showed immuno-positivity for either protein in this case. **b** Patient #4, whose MRI and PET scans are shown in Fig. 1. As in most cases, tumor blood vessels showed strong LAT1 expression (*solid arrow*) along with mild immunostaining of cytoplasm of most neurons (*dashed arrow on the left*). Expression of IDO was variable with some cells showing moderate (*dashed arrow*) and a few cells with strong IDO staining intensity. Original magnification at $\times 20$; scale bar 50 μm

Fig. 4. Variable IDO expression in both tumor neurons (*black solid arrow*) and oligodendrogloma-like cells (*black dashed arrow*) in the DNT of a 3 year-old boy (patient #3). Intense and widespread staining was seen in tumor blood vessels (*white arrow*). Original magnification at $\times 20$; scale bar 50 μm

Clinical, imaging, and immunohistochemical data of 11 patients with epileptogenic dysembryoplastic neuroepithelial tumors

Table 1

Patient No.	Age (y)/Gender	Age at seizure onset (y)	MRI contrast enhancement	Tumor/ Cortex FDG SUV ratio	Tumor/ Cortex AMT SUV ratio	K ratio	VD ratio	IDO cell	IDO vessel	LAT1 cell	LAT1 vessel	Vessel per field
1	6/F	5	No	0.50	1.44	0.97	2.40	0.9	2.4	0.8	3.0	26
2	12/M	2.5	Yes	0.44	1.05	0.88	1.53	n.a.	n.a.	n.a.	n.a.	n.a.
3	3/M	0.5	No	0.55	1.19	0.94	1.95	1.3	3.0	1.0	2.9	5
4	10.3/F	9.5	No	0.45	1.27	1.5	1.55	1.5	1.8	0.9	2.7	16
5	2.5/M	2	No	0.60	1.04	n.a.	n.a.	1.5	2.5	1.3	2.6	8
6	15.5/M	15	No	0.54	1.23	1.07	1.40	1.3	2.3	1.3	2.5	8
7	16.9/F	15	No	0.40	0.87	0.89	0.90	2.3	3.0	1.4	3.0	4
8	15.1/M	15	Yes	0.64	1.08	0.54	1.41	n.a.	n.a.	n.a.	n.a.	n.a.
9	6.1/M	5.6	No	0.29	0.99	1.00	0.97	n.a.	n.a.	n.a.	n.a.	n.a.
10	17.5/M	17.4	Yes	0.56	1.31	1.08	1.57	2.0	2.6	2.8	2.9	9
11	10.9/M	10.3	No	0.40	0.97	n.a.	n.a.	0.3	1.7	0.9	2.0	11

y years; M male; F female; FDG 2-deoxy-2[¹⁸F]fluoro-D-glucose; AMT α[¹¹C]methyl-L-tryptophan; SUV standardized uptake value; K K-complex; VD volume of distribution; n.a. not available

Immunohistochemistry scores (range: 0–3) incorporating staining intensity and density for IDO and the LAT1 are given separately for tumor blood vessels and DNT cell staining in the last four columns. Vessel per field refers to the average number of vessels per field of view, counted in LAT1 stained specimens, where virtually all vessels were stained moderately or strongly