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Evaluation of plasma annexin V levels in children and young adults with solid tumors

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Abstract

Background—Annexin V staining has become a standard approach for identifying cells undergoing apoptosis *in vitro* and can be detected in plasma. We hypothesized that plasma annexin V levels might serve as a clinical marker of tumor burden and cell turnover in children and young adults with solid tumors.

Methods—Nine patients aged 4–22 with newly diagnosed solid tumors were enrolled. Plasma samples were obtained prior to and, in a subset of patients, after initiation of chemotherapy from which annexin V levels were determined by enzyme-linked immunosorbent assay (ELISA).

Results—Three of nine patients had elevated plasma annexin V levels (> 10 ng/mL) at diagnosis and there was poor correlation to LDH levels, a commonly used marker of cell turnover (r = 0.66). Of the five patients with annexin V levels obtained after starting chemotherapy, only one showed an increase over the time period assessed.

Conclusions—Plasma annexin V does not appear to be a useful marker of tumor burden or early response to chemotherapy in children with solid tumors.

Keywords

annexin V; apoptosis; laboratory correlates; pediatric cancers; Sarcoma/soft tissue malignancies

Introduction

One of the earliest indicators of apoptosis, the process of programmed cell death, is externalization of cell membrane phosphatidylserine (1). Annexin V is a cellular protein capable of binding with high-affinity to externalized phosphatidylserine. Annexin V staining has therefore become a standard approach for identifying cells undergoing apoptosis *in vitro* (1).

Previous work has demonstrated that the annexin V protein can be measured in human plasma using enzyme-linked immunosorbent assay (ELISA) techniques. Healthy adults have

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been reported to have average plasma annexin V levels of 0.6 to 6.7 ng/mL (2–5). In almost all studies, healthy volunteers consistently have plasma annexin V levels less than 10 ng/mL. Further studies have shown increased plasma annexin V in patients with trauma, cardiac arrest, stroke, systemic lupus erythematosis, and primary antiphospholipid antibody syndrome (2, 3, 5, 6).

Aside from three patients with lung or renal cancer reported to have increased plasma annexin V levels, no other published reports have described plasma annexin V levels in patients with cancer (2). We therefore initiated this study to determine if children and young adults with newly diagnosed solid tumors have elevated levels of plasma annexin V and to describe changes in these levels after starting chemotherapy. Our hypothesis was that plasma annexin V levels would be elevated at baseline and then increase in response to chemotherapy which would reflect apoptotic activity, increased cell lysis, or both.

Methods

Patients

Patients 1 – 30 years of age were eligible if they had a newly diagnosed, untreated primary solid cancer, excluding lymphoma. Patients were excluded if they had a known inherited or acquired hemolytic anemia, received a blood transfusion within 15 days of study entry or if transfusion was anticipated within 24 hours after study entry as these factors can increase plasma annexin V levels.

All participants or legal guardians provided consent for study participation, with assent obtained as appropriate. The UCSF Committee on Human Research approved the study.

Quantification of Plasma Annexin V Levels

All patients provided a single baseline blood sample in EDTA. For patients receiving neoadjuvant chemotherapy, follow-up samples were requested every 4 hours after the start of chemotherapy until 24 hours had elapsed and then daily if the patient was still admitted to the hospital. Samples were analyzed according to manufacturer recommendation using the Imuclone™ Annexin V ELISA assay (American Diagnostica, Inc). All samples were run in duplicate and high and low controls were validated.

Statistical Methods

The study protocol was designed to enroll 15 patients based on the expected rate of newly diagnosed pediatric solid tumors at our institution over a 12–18 month enrollment period. After reviewing data from the first interim analysis of 9 patients, the study team determined that further evaluation of this putative biomarker was unlikely to be informative and enrollment ended. All statistical analyses were performed using STATA, version 11.

Results

Nine patients were enrolled, with clinical features shown in Table 1. The mean baseline plasma annexin V level was 6.42 ng/mL (range 2.9 – 15.7 ng/mL). Only three of nine subjects had plasma annexin V levels above the upper limit of normal of 10 ng/mL at

baseline. There was poor linear correlation between baseline plasma annexin V level and LDH (r = 0.66; Table 1).

We obtained plasma samples from five patients after the initiation of chemotherapy (Figure 1). Plasma annexin V did not reliably increase in these patients over the time course evaluated. Only one patient showed an increase in annexin V after initiation of chemotherapy to 9.3 ng/mL, still within normal range.

Discussion

Our study, the first to evaluate plasma annexin V levels systematically in patients with cancer, indicates that levels of this protein are not typically elevated in children with solid tumors at initial presentation. In addition, plasma annexin V levels did not increase in response to chemotherapy. These results refute our hypothesis that annexin V levels would be elevated at baseline and then increase in response to chemotherapy.

Based on these results, plasma annexin V will not provide a more precise surrogate marker of cell burden and turnover than the currently accepted marker, LDH (7, 8). A previous study evaluating annexin V levels in adults with acute myocardial infarction noted three patients from the control group who had elevated levels and an underlying cancer diagnosis (2). An additional 21 patients with cancer in the control group did not have elevated annexin V levels, which is consistent with our findings. It remains unknown if plasma annexin V would be a useful marker in patients with hematologic malignancies.

While the current study is limited in size, our early null results suggested that enrollment of additional subjects would not provide additional meaningful data. We conclude that plasma annexin V is not an informative marker of disease burden or response to therapy in children and young adults with solid cancers.

Acknowledgments

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References

1. Lahorte CM, Vanderheyden JL, Steinmetz N, Van de Wiele C, Dierckx RA, Slegers G. Apoptosis-detecting radioligands: current state of the art and future perspectives. Eur J Nucl Med Mol Imaging. 2004; 31:887–919. [PubMed: 15138718]
2. Kaneko N, Matsuda R, Hosoda S, Kajita T, Ohta Y. Measurement of plasma annexin V by ELISA in the early detection of acute myocardial infarction. Clin Chim Acta. 1996; 251:65–80. [PubMed: 8814351]
3. Matsuda R, Kaneko N, Kikuchi M, Chiwaki F, Toda M, Ieiri T, et al. Clinical significance of measurement of plasma annexin V concentration of patients in the emergency room. Resuscitation. 2003; 57:171–7. [PubMed: 12745185]
4. Rand JH, Arslan AA, Wu XX, Wein R, Mulholland J, Shah M, et al. Reduction of circulating annexin A5 levels and resistance to annexin A5 anticoagulant activity in women with recurrent spontaneous pregnancy losses. Am J Obstet Gynecol. 2006; 194:182–8. [PubMed: 16389029]
5. Van Heerde WL, Reutelingsperger CP, Maassen C, Lux P, Derksen RH, De Groot PG. The presence of antiphospholipid antibodies is not related to increased levels of annexin A5 in plasma. J Thromb Haemost. 2003; 1:532–6. [PubMed: 12871462]

6. Peetz D, Hafner G, Blankenberg S, Peivandi AA, Schweigert R, Brunner K, et al. Annexin V does not represent a diagnostic alternative to myoglobin for early detection of myocardial infarction. Clin Lab. 2002; 48:517–23. [PubMed: 12389712]

7. Cohn SL, Pearson AD, London WB, Monclair T, Ambros PF, Brodeur GM, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. J Clin Oncol. 2009; 27:289–97. [PubMed: 19047291]

8. Cotterill SJ, Ahrens S, Paulussen M, Jurgens HF, Voute PA, Gadner H, et al. Prognostic factors in Ewing's tumor of bone: analysis of 975 patients from the European Intergroup Cooperative Ewing's Sarcoma Study Group. J Clin Oncol. 2000; 18:3108. [PubMed: 10963639]

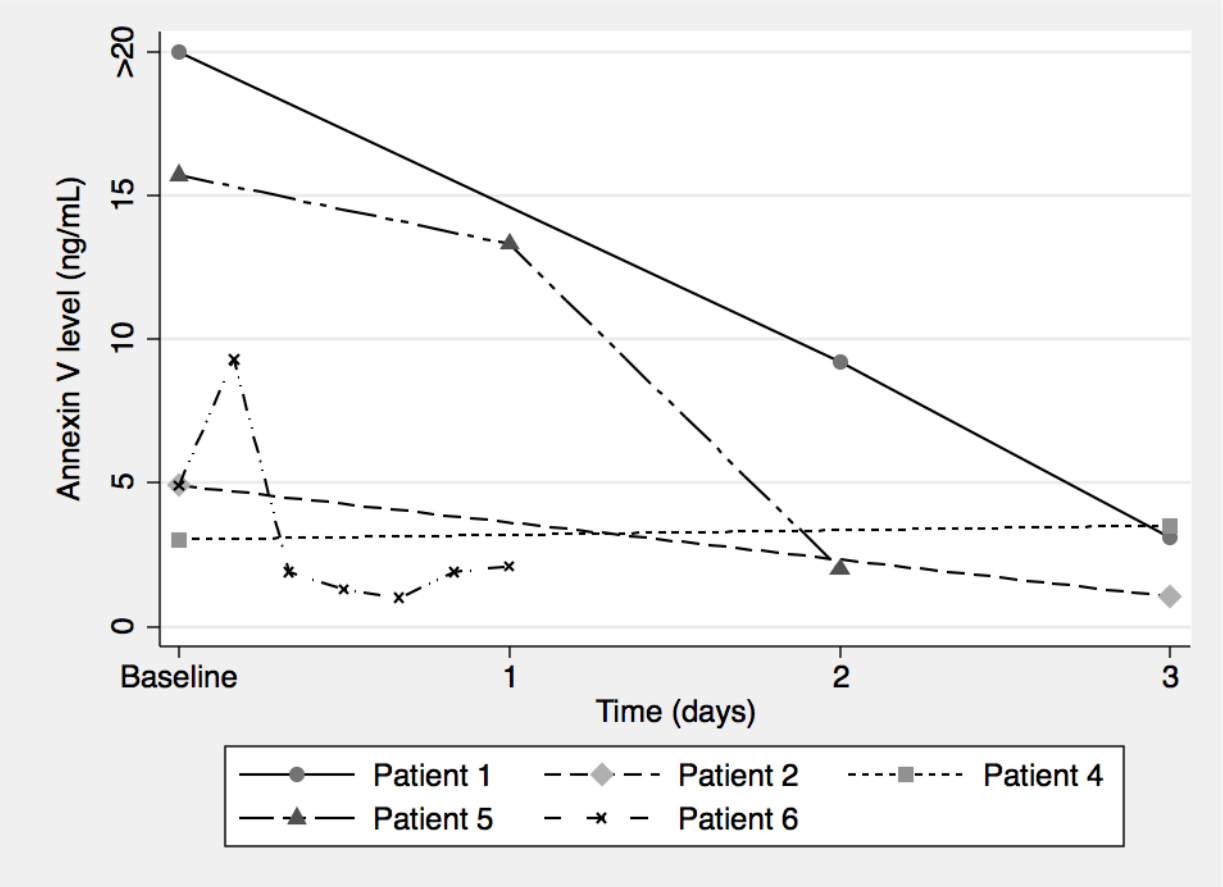

Figure 1.
Serial plasma annexin V levels in 5 patients at baseline (hour 0) and after initiation of neoadjuvant chemotherapy.

Table 1

Patient characteristics and baseline laboratory values in 9 patients with solid malignancies.

Patient	Age at diagnosis (yr)	Diagnosis	Metastatic status	Plasma Annexin V at baseline (ng/mL)	LDH at baseline (IU/L)
1	17	Osteosarcoma	Local	Out of range, >20	266
2	4	Ewing sarcoma	Local	4.91	188
3	4	Wilms tumor	Local	3.12	310
4	22	Epithelioid sarcoma	Metastatic	3.03	116
5	8	Mesenchymal chondrosarcoma	Metastatic	15.7	397
6	21	Osteosarcoma	Metastatic	4.9	294
7	16	Ewing sarcoma	Local	4.6	184
8	16	Nasopharyngeal carcinoma	Metastatic	4.1	340
9	10	Ewing sarcoma	Local	11.2	376