

Letter to the Editor

^{99m}Tc-heparin: A new approach to detection of eosinophilic esophagitis-associated inflammation

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In eosinophilic esophagitis (EoE), eosinophils infiltrate the esophagus, where they degranulate, releasing cytotoxic granule proteins, including major basic protein (MBP-1). However, the variability of eosinophil involvement presents a diagnostic challenge to the gastroenterologist because current diagnosis requires invasive esophagogastroduodenoscopy with a minimum of 5 biopsies, representing less than 0.02% of the esophageal surface. The disease is known to be patchy, which may result in underdiagnosis, particularly for mild to moderately involved patients.^{1,2} To understand this disease, improved, preferably non-invasive, methods to characterize the severity of eosinophil-associated inflammation are required.

We propose a novel strategy to localize cationic eosinophil granule proteins by using anionic heparin radiolabeled with technetium-99m (^{99m}Tc). Proteins deposited in the esophagus after eosinophil degranulation, such as MBP-1, are primary candidates, because they are absent in the normal esophagus. Here, we hypothesize that ^{99m}Tc-heparin binds avidly to cationic eosinophil granule proteins, including MBP-1, and can be used to determine the presence of eosinophil products in the diseased esophagus by using single photon emission computed tomography (SPECT) imaging.³ Previous studies have shown that MBP-1 is deposited in the esophageal tissues and is available for binding.^{4,5} Heparin is a promising candidate because it has a very high negative charge density and is not absorbed from the gastrointestinal tract.⁶⁻⁸

Solutions of stannous chloride (40 mg/mL, Sigma, St Louis, Mo; 243523) were prepared in deionized water under flowing nitrogen. A 0.5 mL aliquot was filtered and mixed with 1.00 mL of NaCl (1.00 mol/L) plus 150 mg of preservative-free heparin (10,000 IU/mL). Approximately 100 mCi of freshly eluted ^{99m}Tc was added and mixed for 30 minutes at room temperature. Aliquots containing approximately 10 mCi of ^{99m}Tc and 20 mg of heparin were removed for tissue experiments. The labeling efficiency, measured by paper chromatography Whatman #31 with acetone, showed more than 97% binding of heparin to ^{99m}Tc. Radiolabeled heparin was also analyzed by using Sephadex G25 column chromatography (HiTrap 5 mL desalting columns, GE Healthcare, Pittsburgh, Pa; 17140801) with 0.15 mol/L NaCl as the elution buffer, and 1.0 mL fractions were collected. Radioactivity eluted at fraction 4 (free ^{99m}Tc elutes at fraction 13), demonstrating that all the ^{99m}Tc eluted at the void volume and confirming that there is negligible unbound ^{99m}Tc in the radiolabeled heparin. The stability of ^{99m}Tc-heparin in an acidic environment was tested by diluting in artificial gastric juice (Carolina Biological Supply Company, Burlington, NC; 864603) and showing by using both paper chromatography and Sephadex G25 that its properties were unaltered.

Esophageal biopsies were collected from normal subjects and patients participating in a prior elemental diet study (IRB 00040035 University of Utah) and kept frozen at -70°C. Biopsies from each patient were selected for histologic analysis for eosinophil density or radiolabeling. Biopsies from normal

TABLE I. Radioactivity measurements, histologic data, and the SPECT count of EoE versus control samples

Sample no.	Disease state	Eosinophil loads (eos/hpf)*	Activity (μCi)	SPECT count
1	Active EoE	78	122.5	59,634
2	Active EoE	51	87.1	40,474
3	Active EoE	53	75.7	46,457
4	Active EoE	46	98.1	59,923
5	Active EoE	78	52.4	31,780
6	Resolved EoE	10	100	47,048
7	Resolved EoE	0	19.9	13,202
8	Resolved EoE	0	27.7	17,518
9	Normal	0	21.4	17,740
10	Normal	0	41.3	25,766

*Eosinophil load is based on 10 hpf counts.

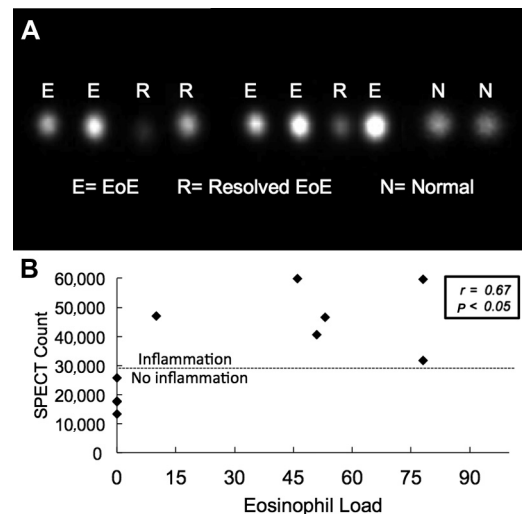


FIG 1. A, Two-dimensional planar SPECT image of biopsies from normal, active EoE, and resolved EoE patients. From right, the samples are from patient no. 5, 3, 7, 6, 4, 2, 8, 1, 9, and 10, respectively. Multiple SPECT images were combined to make panel A. **B,** SPECT count of biopsies as a function of eosinophil counts from the same patient.

subjects and patients with EoE who resolved their esophageal eosinophilia under the elemental diet were chosen as negative controls. Tissues from normal, resolved EoE, and active EoE patients were incubated with ^{99m}Tc-heparin, washed in buffered saline, and imaged by using SPECT.

The activity of radiolabeled biopsy tissues was measured by using a dose calibrator (Capintec model CRC-15R). Two-dimensional planar static images of the radiolabeled biopsies were obtained using an Ecam SPECT instrument (Siemens, Munich, Germany). The images were acquired for 3 minutes each by using a 1.0 magnification, 256 by 256 matrix size. Correlation between eosinophil densities and SPECT counts obtained by 2-dimensional imaging were evaluated by Spearman rank correlation analysis.

Table I presents the SPECT counts for each biopsy, summarizing its radioactivity and eosinophil density. Histopathologic analyses confirmed that only samples 1 to 5 had eosinophil levels of 15 or more eosinophils/high-power field (hpf). The images (Fig 1)

suggest that active EoE cases can be differentiated from resolved EoE cases by using this technique. Indeed, the Spearman rank correlation analysis finds a monotonic correlation between eosinophil density and SPECT count ($r = 0.67$; $P < .05$). Remarkably, even though the eosinophil densities are derived from the same patient but not from the same biopsy specimen used for SPECT imaging, tissues from inflamed patients displayed significant binding to ^{99m}Tc -heparin that correlates with eosinophil density.

These proof-of-principle experiments clearly demonstrate that, first, heparin can be labeled with ^{99m}Tc in the presence of fresh stannous chloride without any other chelating agent. Second, eosinophil density correlates with ^{99m}Tc emission as measured both in a well counter and by SPECT. Third, eosinophil-enriched tissues can be distinguished from eosinophil-poor tissues by using the ^{99m}Tc -heparin conjugate and SPECT quantitation. Yet, the analysis finds that 15 eosinophils/hpf may not be the best cutoff to distinguish granule protein deposition. For example, our analysis finds significant SPECT counts at 10 eosinophils/hpf (see sample 6 in Table I). Some variation around the nominal cutoff of 15 eosinophils/hpf is not unexpected because eosinophil presentation in EoE is known to be patchy, the eosinophil density and SPECT count are not from identical biopsies but from the same patient, and some eosinophils may have degranulated completely leaving only the MBP-1 signature.² While the full set of all proteins that bind ^{99m}Tc -heparin remains unclear, we assume that they are likely derived from eosinophils because of the cationicity of the eosinophil granule proteins and their known abilities to bind heparin. Other products derived from mast cells, such as mast cell tryptase, may also bind ^{99m}Tc -heparin because tryptase is known to bind to heparin.⁹ However, prior studies discourage the belief that tryptase deposition is widespread.¹⁰

This is the first and only study to experimentally evaluate radiolabeled contrast agents for the detection of EoE-associated inflammation. Further studies are underway to rigorously validate this technique as an assay for eosinophil involvement and to evaluate oral administration of ^{99m}Tc -heparin in the presence of a mucosal layer and esophageal peristalsis. Although radioactivity offers considerable sensitivity as demonstrated, the binding of the heparin conjugate to the EoE-associated inflammation will likely depend on the esophageal residence time (or passage rate), which will have an impact on the sensitivity of the assay. However, we believe that the current findings are important because they provide a new avenue for the quantification of inflammation. If successful, this strategy will provide unprecedented insight into the specific involvement of eosinophils and their granule proteins within the esophagus of patients with EoE, which will create improved opportunities to understand the disease pathogenesis and treatment efficacy.

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REFERENCES

- Gonsalves N, Policarpio-Nicolas M, Zhang Q, Rao MS, Hirani I. Histopathologic variability and endoscopic correlates in adults with eosinophilic esophagitis. *Gastrointest Endosc* 2006;64:313-9.
- Saffari H, Peterson KA, Fang JC, Teman C, Gleich GJ, Pease LF. Patchy eosinophil distributions in an esophagectomy specimen from a patient with eosinophilic esophagitis: implications for endoscopic biopsy. *J Allergy Clin Immunol* 2012;130:798-800.
- Swaminathan GJ, Mysza DG, Katsamba PS, Ohunki LE, Gleich GJ, Acharya KR. Eosinophil-granule major basic protein, a C-type lectin, binds heparin. *Biochemistry* 2006;44:14152-8.
- Kephart GM, Alexander JA, Arora AS, Romero Y, Smyrk TC, Talley NJ, et al. Marked deposition of eosinophil-derived neurotoxin in adult patients with eosinophilic esophagitis. *Am J Gastroenterol* 2010;105:298-307.
- Talley NJ, Kephart GM, McGovern TW, Carpenter HA, Gleich GJ. Deposition of eosinophil granule major basic protein in eosinophilic gastroenteritis and celiac disease. *Gastroenterology* 1992;103:137-45.
- Hirash J, Warkentin TE, Shaughnessy SG, Anand SS, Halperin JL, Raschke R, et al. Heparin and low-molecular-weight heparin: mechanisms of action, pharmacokinetics, dosing, monitoring, efficacy, and safety. *Chest* 2001;119:64S-94S.
- Laforest MD, Colas-Linhart N, Guiraud-Vitau F, Bok B, Bara L, Samama M, et al. Pharmacokinetics and biodistribution of technetium 99m labelled standard heparin and a low molecular weight heparin (enoxaparin) after intravenous injection in normal volunteers. *Br J Haematol* 1991;77:201-8.
- Kulkarni PV, Parkey RW, Wilson JE III, Lewis SE, Buja LM, Bonte FJ, et al. Modified technetium-99m heparin for the imaging of acute experimental myocardial infarcts. *J Nucl Med* 1980;21:117-21.
- Johnson DA, Barton GJ. Mast cell tryptases: examination of unusual characteristics by multiple sequence alignment and molecular modeling. *Protein Sci* 1992;1:370-7.
- Perez GL, Peters MS, Reda AM, Butterfield JH, Peterson EA, Leiferman KM. Mast cells, neutrophils, and eosinophils in prurigo nodularis. *Arch Dermatol* 1993;129:861-5.