

Scintigraphic Detection of Splenosis and Accessory Spleen by Using Tc-99m Labelled Denatured Red Blood Cells

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ABSTRACT

Objective: To evaluate the usefulness of Tc-99m-labelled denatured red blood cell scintigraphy (DRBCS) in the detection of splenosis and accessory spleen.

Methods: This retrospective study reviewed 7 patients who were suspected of splenosis and accessory spleen during 2007-2014. Six patients had undergone total splenectomy before the DRBCS study. Multi-planar, SPECT and SPECT/CT images of DRBCS were reviewed by visual analysis. Image findings were correlated with other imaging modalities and clinical presentations at follow-up.

Results: DRBCSs were positive in 6 patients; 5 splenosis and 1 accessory spleen. A single lesion was detected in each patient. Locations were in splenic bed (5 patients) and in pelvic cavity (1 patient). Mean lesion size was 3.7 +/- 2.4 cm. SPECT or SPECT/CT imaging could eliminate false-negative results in 2 patients. Lesion uptake intensity in 4 patients was higher than in the liver. Two patients had subsequently undergone resection of the lesions and pathological examinations confirmed the diagnosis of splenosis and accessory spleen. Sensitivity of DRBCS was comparable with other imaging modalities. However, DRBCS was more specific for the splenic tissue.

Conclusion: DRBCS is useful for the detection of splenosis and the accessory spleen. Detection sensitivity of DRBCS is better than ultrasonography(US). DRBCS is more specific to the splenic tissue, compared with US and CT. Furthermore, SPECT/CT imaging increases sensitivity, lesion localization and characterization. Clinical impact of DRBCS is apparent including pre-surgical localization, elimination of additional follow-up imaging and invasive procedure.

Keywords: Accessory spleen; denatured red blood cell; immune thrombocytopenia; splenosis; spleen scintigraphy (Siriraj Med J 2019;71: 143-149)

INTRODUCTION

The spleen is the largest lymphoid organ which plays an important role in regulating immune cells and mediators, removing circulating microorganisms through phagocytosis and filtering damaged cells and foreign material.¹ Splenosis is the acquired condition from autotransplantation of

splenic tissue that usually occurs after splenic trauma or splenectomy. In contrast, the accessory spleen is a congenital anomaly of the spleen.² These conditions can present a diagnostic challenge and can be particularly difficult to distinguish by using conventional imaging modalities such as ultrasonography (US), computed

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tomography (CT) and magnetic resonance imaging (MRI). Improved characterization may be obtained by using superparamagnetic iron oxide as a contrast-enhancing agent in MRI.³ In contrast, spleen-specific imaging using Tc-99m labeled denatured red blood cells scintigraphy (DRBCS) is a more simple technique that can definitely distinguish splenic tissue from other benign/malignant non-splenic origin according to intrinsic physiologic uptake of DRBC by functioning splenic tissue.

DRBCS was introduced as a spleen-specific tracer since 1967 and has been shown to be of value in the detection of both native spleen and accessory spleen.⁴ Clinical indications for DRBCS include congenital/functional asplenia, accessory spleen, space-occupying lesion in the spleen, refractory immune thrombocytopenia (ITP) after splenectomy, and thoracic/abdominopelvic mass suspected for the accessory/ectopic spleen.

The objective of this study was to evaluate the usefulness of DRBCS in the detection of splenosis and accessory spleen by 1) reporting the DRBCS findings and compare with the other imaging modalities and 2) investigating the clinical impact of DRBCS in the patients with suspected splenosis and accessory spleen.

MATERIALS AND METHODS

This retrospective study was approved by The Institutional Review Board (Si 085/2015). We searched our internal clinical database for the time period between 2007 and 2014 and identified 8 patients who had undergone DRBCS from our Nuclear Medicine Unit. The inclusion criteria were the patients who had undergone DRBCS for the evaluation of splenosis or accessory spleen. We excluded 1 patient who had undergone DRBCS for the evaluation of the space-occupying lesion in the native spleen. Our final study cohort comprised of 7 patients.

DRBCS protocol

Preparation of Tc-99m DRBC

1. Reconstitute stannous kit with normal saline and intravenously inject to a patient.
2. After 20-30 min, draw blood of 10 ml into a heparinized syringe.
3. Using a lead glass shielding, add 20 mCi of $\text{Na}^{99\text{m}}\text{TcO}_4$ to the heparinized whole blood.
4. After 5 min, incubate the tube in a constant-temperature water bath at $50 \pm 1^\circ\text{C}$ for 20 minutes with periodical shaking.
5. Centrifuge, wash and reconstitute with NSS.
6. Check the percentage of DRBC and re-inject to the patient. Denaturation of RBC was technically successful in all patients. In every patient, no complications occurred

after the re-injection. The preparation was tested in one of our research team as a healthy volunteer and the images shown a normal distribution (Fig 1).

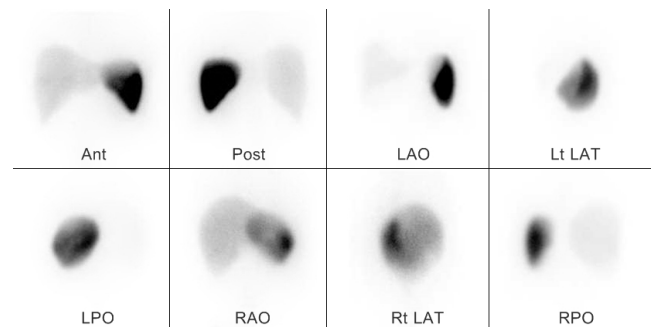


Fig 1. DRBCS of a normal volunteer. The uptake in spleen is homogeneously intense. Liver uptake is faint. No marrow uptake is observed.

Abbreviations: Ant: Anterior; Post: Posterior; LAO: Left anterior oblique; Lt LAT: Left lateral; LPO: Left posterior oblique; RAO: Right anterior oblique; Rt LAT: Right lateral; RPO: Right posterior oblique.

DRBCS imaging protocol

Scintigraphy began 30 minutes after the injection of DRBC, using a gamma camera equipped with a high-resolution, low-energy collimator. Static planar images of the abdomen were obtained in anterior, posterior, both anterior and posterior oblique (LAO, LPO, RAO, RPO), and both lateral views in all patients with the same imaging acquisition protocol. After planar imaging, single photon emission computed tomography (SPECT) and SPECT/CT imaging was obtained over the abdomen in the selected patients. One patient (patient No.6) was obtained planar imaging only. Two and 4 patients were performed SPECT and SPECT/CT imaging sequentially after the planar scans.

Image analysis

DRBCS images were retrospectively analyzed on a printed film or electronic archiving system by a nuclear medicine physician who was unaware of other clinical data. The following findings were determined by visual analysis: (1) the presence or absence of abnormal tracer uptake suggesting splenosis or accessory spleen; (2) the intensity of uptake (compared with the liver uptake); and (3) the location of the abnormal uptake lesion(s). Lesion size was measured on the SPECT/CT images. In patients without SPECT/CT imaging, lesion size was evaluated by other imaging modalities. Other imaging data (the US in 4 patients and contrast-enhanced CT in 1 patient) were reviewed by a radiologist. The DRBCS

findings were compared with other imaging modalities and pathological findings as a gold standard.

Statistical analysis

The analyses were performed by using statistical software (SPSS version 18.0; SPSS Inc., Chicago, IL, USA). All continuous (quantitative) data, including the patient's age and lesion size, were summarized.

RESULTS

Four patients were male and 3 were female. The median age was 31 years (range 5 – 70 years). Three patients were children; age ranged from 5-8 years. Six patients had undergone total splenectomy prior to the DRBCS; 1 of these had undergone 2 sessions of splenectomy according to refractory ITP (patient No. 4). None of the study population had a history of abdominal trauma. The clinical indications for performing DRBCS were to localize residual splenic tissue causing refractory ITP post-splenectomy (5 patients), to differentiate between splenosis and metastatic cancer (1 patient) and to evaluate pre-operatively for the biopsy confirmed accessory spleen (1 patient). Time intervals between splenectomy and DRBCS were within 1 year in 3 patients, 3 years in 1 patient and 33 years in 2 patients.

Of the 5 patients with refractory ITP, 1 patient had secondary ITP due to HCV infection (patient No. 3), 1 patient had acute ITP with a subdural hematoma and had undergone emergency splenectomy (patient No. 1) and the rest were chronic ITP patients. All 5 patients were classified as 'no response' to treatment based on the criteria of the International Working Group (a platelet count $<30 \times 10^9/L$ or less than 2-fold increase in platelet count from baseline or the presence of bleeding).⁵ Patient characteristics, clinical information, image acquisitions/ findings, and pathological results, are listed in [Table 1](#).

DRBCS image quality was excellent in 5 scans. A patient (No. 3) showed an abnormal homogeneous increased tracer uptake in the liver and bone marrow. Specifically, the uptake in the residual spleen was less than in the liver. In this case, planar imaging gave a false negative result, but SPECT/CT imaging could demonstrate the residual spleen ([Fig 2](#)). Another patient (No. 2) had uptake in splenosis less than in the liver, but no marrow uptake.

The scan was negative in a patient with refractory ITP after an emergency splenectomy for acute ITP with subdural hematoma ([Fig 3](#)). The rest 6 scans were positive for residual spleen or accessory spleen. A single positive lesion was detected in all patients. Median lesion size was

2.8 cm (range 1.4-7.4 cm). Location of the all residual spleens was in the left upper quadrant of the abdomen ([Fig 4](#)). The accessory spleen was in the pelvic cavity along with the lobulated-shaped native spleen in the splenic bed. In this patient, the accessory spleen was subsequently removed and pathologically confirmed splenic tissue ([Fig 5](#)).

SPECT and SPECT/CT imaging were subsequently obtained after the routine multi-planar views in 2 and 4 patients, respectively. SPECT/CT could identify the residual spleen in a patient with negative multi-planar scan (patient No. 3). This patient had crescent-shaped residual spleen in the location that almost attached to the left liver lobe, which was misinterpreted as the elongated left liver lobes in the planar images. However, the lesion was clearly demonstrated in the 3-D imaging by SPECT/CT scanning.

Two out of 7 patients had undergone resection of the residual/accessory spleen following the positive DRBCS (patient No. 4 and 7). The pathological results confirmed splenic tissue in both cases. However, there was no rising of the platelet level after the surgery in patient No. 4 and she needed long-term treatment with corticosteroid. Patient No. 7 required resection of the accessory pelvic spleen due to compression effect to the bladder. His urinary incontinence subsided after the surgery. DRBCS prevented futile investigation/ surgery in patient No. 5, who was confirmed to have a residual spleen, not a focus of metastatic colon cancer.

Removal of the residual spleen was not undertaken in 3 patients with refractory ITP despite the positive DRBCS results (patient No. 2, 3 and 6). One of them (patient No. 6) had a spontaneous rising of the platelets to the normal range without any specific treatment. Patient No. 3 responded to corticosteroid and Eltrombopag. Nonetheless, patient No. 2 as well as patient No. 1 (whose DRBCS result was negative), had no response to the medical treatment.

Comparison of DRBCS and other imaging modalities results was summarized in [Table 2](#). The detection sensitivity of DRBCS is better than US. DRBCS is more specific to spleen tissue, compared with US and CT.

DISCUSSION

Splenosis is the acquired benign condition. It is estimated to occur in 26%-67% of the patients after traumatic or iatrogenic splenic parenchymal rupture.² The mechanism is the autotransplantation of the spillage cells to the nearby body cavity or hematogenously spreading of the cells and implanting to the remote sites.⁶ The most common location of splenosis is in the left upper quadrant

TABLE 1. Baseline patient characteristics and main results.

No.	Sex/ Age (years)	Splenectomized	Indication	Planar	SPECT or SPECT/CT ¹	Intensity ²	Lesion number	Lesion size (cm) ³	Location	Pathology
1	M/31	+	Refractory ITP	-	-	0	0	NA	NA	NA
2	F/5	+	Refractory ITP	+	+	1	1	2	Splenic bed	NA
3	F/54	+	Refractory ITP	-	+	1	1	4.7	Splenic bed	NA
4	F/44	+	Refractory ITP	+	+	3	1	1.4	Splenic bed	Confirmed
5	F/70	+	DDx. Metastasis vs. splenosis	+	+	3	1	2.8	Splenic bed	NA
6	M/8	+	Refractory ITP	+	NA	3	1	NA	Splenic bed	NA
7	M/7	-	Pelvic mass	+	+	3	1	7.4	Pelvic	Confirmed

Abbreviations: M: Male; F: Female; ITP: Immune thrombocytopenia; DDx: Differential diagnosis; SPECT: Single photon emission computed tomography; CT: Computed tomography; NA: Not available.

¹ SPECT only in patient No. 1 and 2; SPECT/CT in patient no. 3-5 and 7

² Intensity of uptake, compared to the liver: 0 = no uptake, 1 = less than the liver, 2 = equal to the liver, 3 = greater than the liver

³ Maximal diameter; measured on SPECT/CT images. Patient No. 2 was measured on ultrasonography (performed SPECT without CT)

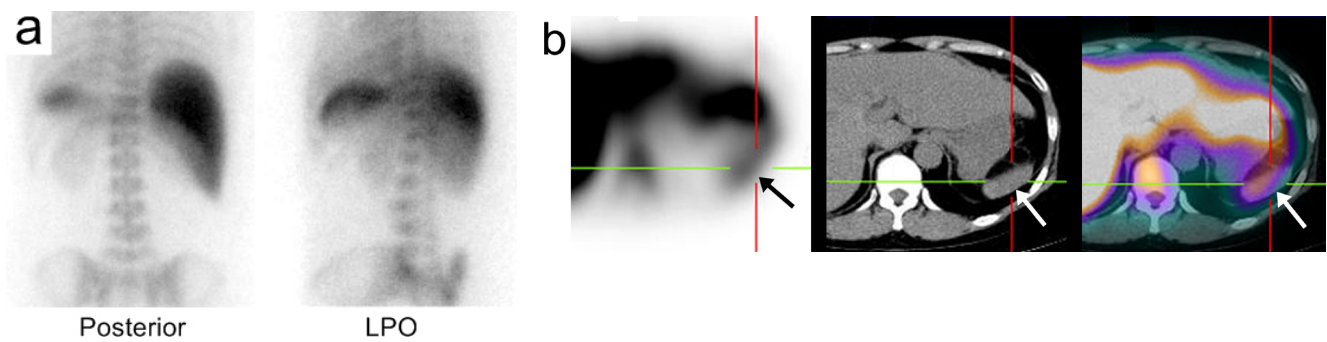


Fig 2. Abnormal distribution and false-negative planar scan. A 54-year old female with HCV-related refractory ITP status post splenectomy 33 years ago (Patient No. 3). DRBCS planar images (a) show abnormally intense uptake in the liver and diffused marrow uptake. Planar images are negative. DRBCS SPECT/CT (b) images reveal a faint uptake corresponding with a curvilinear soft tissue locates beneath the posterior aspect of the left hemidiaphragm, measured about 1.5 x 4.7 x 1.6 cm. that suggestive of splenosis.

Abbreviation: LPO: Left posterior oblique.

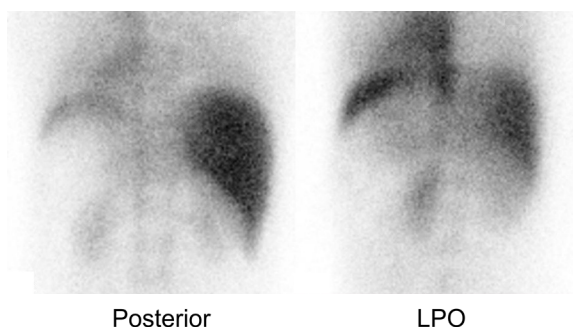


Fig 3. Negative study. A 31-year old man with acute ITP with subdural hematoma status post emergency splenectomy (Patient No. 1). DRBCS was sent due to no platelet response after splenectomy. DRBCS images show no evidence of residual splenic tissue. He has been treated with high-dose prednisolone, but no platelet response.

Abbreviation: LPO: Left posterior oblique.

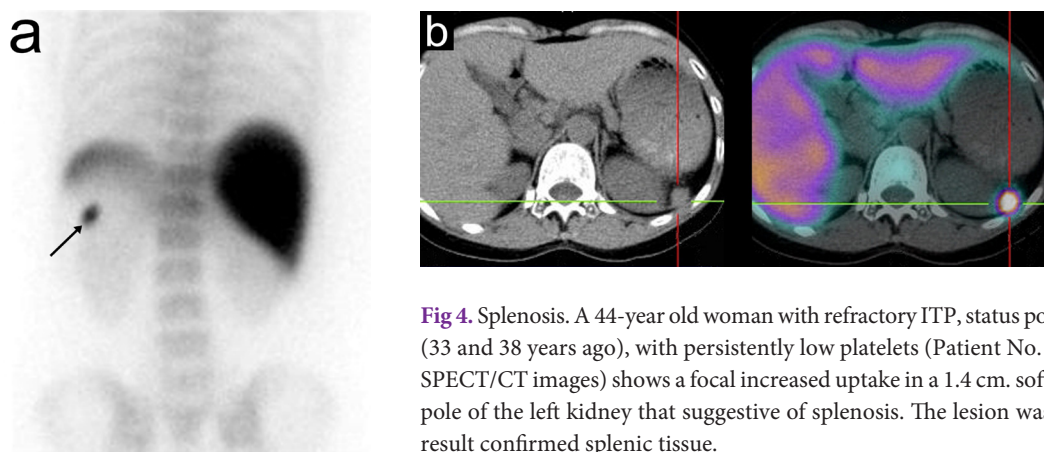


Fig 4. Splenosis. A 44-year old woman with refractory ITP, status post 2 sessions of splenectomy (33 and 38 years ago), with persistently low platelets (Patient No. 4). DRBCS (a Planar and b SPECT/CT images) shows a focal increased uptake in a 1.4 cm. soft tissue mass near the upper pole of the left kidney that suggestive of splenosis. The lesion was resected and pathological result confirmed splenic tissue.

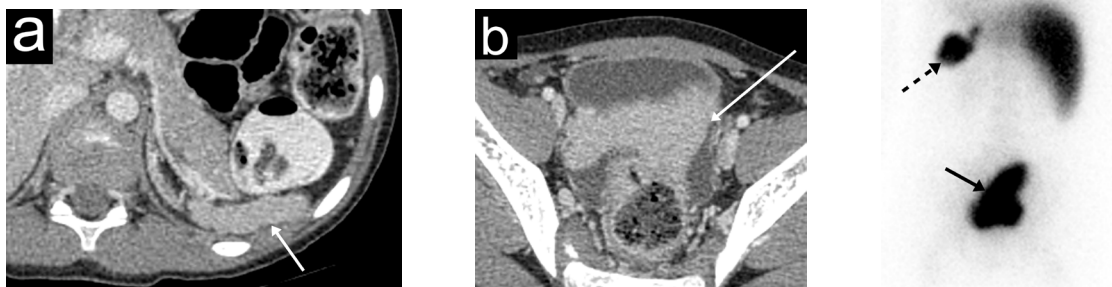


Fig 5. Accessory spleen in the pelvic cavity. A 7-year old boy presented with urinary incontinence (Patient No. 7). The CT shows the small spleen (a) and a lobulated homogeneous enhancing soft tissue mass in the pelvic cavity (b). The differential diagnosis includes lymphoma, germ cell tumor and other mesenchymal tumors. The pelvic mass was biopsied and revealed splenic tissue. DRBCS (c) was sent for pre-operative evaluation. There is increased uptake in the pelvic mass, size about 7.4 x 2.6 cm. (arrow) as well as in the lobulated shaped spleen in the left upper quadrant (dot arrow). He had undergone removal of the accessory spleen and the pathological result confirmed the diagnosis.

TABLE 2. Comparison of DRBCS and other imaging modalities.

No.	DRBCS	Other imaging modalities	
4	Positive	US	Negative
5	Positive	US	Negative
7	Positive	US	Positive with concordant result
8	Positive	US	Positive with inconclusive nature of the lesion
6	Positive	CECT	Positive with inconclusive nature of the lesion

Abbreviations: US: ultrasonography; CECT: contrast enhanced computed tomography.

of the abdomen. Usually, it is asymptomatic, incidentally detected and requires no treatment, because it may have some immunologic value and microorganism filtering function.³ However, in patients with ITP, splenosis leads to failure of the platelet response after splenectomy, owing to the presence of functioning splenic tissue. Detection and removal of the residual functioning splenic tissue are essential for the treatment in this patient group.

DRBCS is the recommended imaging modality for the detection of splenosis.⁶ The uptake mechanism is spleen-specific, based on the sequestration of the DRBC by the pulp region of splenic tissue⁷. The sensitivity of DRBCS is higher than that of US, CT^{6,8}, Tc-99m labeled sulfur colloid scintigraphy^{6,9,8}, and laparoscopy.¹⁰ Moreover, DRBCS is non-invasive, technically simple and able to obtain whole-body imaging within a single injection. Despite the superiority of DRBCS, it is not frequently reported and studied. In our institution, only 8 patients and 1 healthy volunteer performed DRBCS since we established this test in 2007.

DRBCSs are able to detect both splenosis and accessory spleen. In the literature, splenosis is usually observed in multiple¹¹, but we detected a solitary lesion in every case. Our findings confirmed that the most common location of splenosis is in the left upper quadrant. We found a patient with pelvic accessory spleen, which is a very rare condition. In literature, accessory spleens were found in splenic hilum in 75% of cases, and less in the gastrosplenic ligament, splenorenal ligament and pancreatic tail. The development of an accessory spleen presumably involves multifocal embryonic origins. In most reports, spleens detected in pelvic regions are from a different origin, the wandering spleens, which are the abnormal position of the spleen caused by splenic ligament laxity.¹²

The sensitivity of DRBCS was confirmed to be higher than other modalities such as US, CT, and Tc-99m

sulfur colloid scan, especially for the small lesion and in the unusual location.^{9,8,13,4} The differential diagnosis for intraabdominal soft tissue nodule seen on CT images including peritoneal metastasis, lymphadenopathy, lymphoma, pancreatic mass, endometriosis, uterine mass, teratoma and splenosis.^{6,14} DRBCS was more specific to splenic tissue than CT. In our patients, DRBCS ruled out the metastatic nodule and teratoma, so futile biopsy or surgery could be avoided.

In the current study, DRBCSs were positive in 6 (85.7%) patients. Of these, 1 was negative on the planar scan but was clearly positive on the SPECT/CT scan. The planar scan was negative due to the superimposition of splenic tissue by the liver tail. The problem was eliminated by SPECT/CT which provided attenuation correction, three-dimensional view, and higher contrast resolution. Moreover, lesion characterization and anatomical localization using CT technique are important for the patients who planned for pre-surgery. The superior sensitivity of SPECT or SPECT/CT imaging was in concordance with the previous study.¹⁵ However, another study showed no contribution of SPECT to the planar imaging.¹⁶

In this study, only 1 out of 4 patients with refractory ITP and positive DRBCS results had undergone removal of the splenic tissue. However, that patient still suffered from persistently low platelets after the surgery. On the other hand, another patient had spontaneously increased platelet level to the normal range without any surgical or medical treatment. Because DRBCS is spleen-specific, the false-positive result is not possible and have never been reported in the literatures.¹⁶ Post-surgical failure could be described by several factors such as rapid regeneration of the residual splenic tissue, the presence of IgG against platelet and other sites of platelet sequestration.¹⁷

There were limitations to this study. Firstly, this was retrospective which could have many uncontrolled factors. Secondly, it contained a small sample size. Thirdly, we

used different gamma cameras with different scanning parameters. And lastly, pathological confirmation was not done in every case.

CONCLUSION

DRBCS is useful for the detection of splenosis and the accessory spleen. The detection sensitivity of DRBCS is better than US. DRBCS is more specific to spleen tissue, compared with US and CT. Furthermore, SPECT/CT imaging increases sensitivity, localization and lesion characterization. Clinical impact of DRBCS is apparent including pre-surgical localization, elimination of additional follow-up imaging and invasive procedure.

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Conflict of Interest: The research authors (Apichaya Claimon and Shanigarn Thiravit) received a Chalermprakiat grant from the Faculty of Medicine, Siriraj Hospital, Mahidol University. All other authors (Napaporn Tojinda, Ninmanee Taweewatanasopon, Pornphit Boonkhon, Boontham Amornkitticharoen, and Sirilak Wiriyakradecha) declare that they have no conflict of interest.

Ethical Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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