

The value of computed tomography in determining the percentage of hepatosteatosis: An experimental animal study

Karaciğer yağlanması yüzdesinin tespit edilmesinde bilgisayarlı tomografinin önemi: Deneysel hayvan çalışması

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Background and Aims: To analyze the degree of hepatosteatosis and computed tomography findings experimentally using the mouse liver to create computed tomography indexes for determining the degree of hepatosteatosis of the donor liver. **Materials and Methods:** Forty Swiss Albino mice were obtained. Whole body computed tomography scans were obtained by Picker MxTwin four-slice multidetector computed tomography device. To induce hepatosteatosis, the mice were fed by oral gavage with 0,06 mL CCl₄ plus 0,04 mL corn oil, totally 0,1 mL, in 2500 mg/kg/day CCl₄ dosages. The study was continued for four weeks. The computed tomography density measurements were obtained by taking the average of three different 0,5 cm² areas in every organ. Data were compared with pathology. The livers, spleens, kidneys, and paravertebral muscles of the mice were removed. Five-micron thick slices from the paraffin blocks were mounted to slides. An interactive image analysis system (Aequitas, Dynamic Data Links) was used to measure the degree of steatosis in the liver specimens. For each specimen, the proportion of liver with steatosis was determined. Results were assessed statistically. **Results:** A reduction of 40 Hounsfield units in liver density indicates 30% hepatosteatosis. There was a statistically significant correlation between the liver/spleen, liver/kidney and liver/muscle ratios and the histomorphometric hepatosteatosis percentage. When the liver/spleen ratio is ≤0,32, the critical value for the transplantation of ≥30% hepatosteatosis must be doubted. **Conclusions:** With the measurement of the liver/spleen ratio, the total hepatosteatosis percentage can be calculated according to a formula, which we introduce herein, based on our experimental study.

Keywords: Liver transplantation, fatty liver, multidetector computed tomography, experimental animal use, complications

INTRODUCTION

The prevalence of hepatosteatosis in cadaveric and living donors was determined as approximately 20%. Five to six percent of cadaveric livers were rejected because of hepatosteatosis. When the steatotic livers are used, both

Giriş ve Amaç: Verici karaciğerinin yağlanma derecesinin tespit edilmesi için fare karaciğerlerini kullanmak suretiyle bilgisayarlı tomografi indeksleri oluşturarak deneysel olarak yağlanma derecesinin ve bilgisayarlı tomografi bulgularının analizi. **Gereç ve Yöntem:** Kırk adet Swiss Albino fare elde edildi. Picker MxTwin marka 4 kesitli multidetektör bilgisayarlı tomografi cihazı kullanılarak farelerin tüm vücut tomografik incelemeleri sağlandı. Fareler 0,06 mL CCl₄M ve buna ek olarak 0,04 mL mısırözü yağı ile toplam 0,1 mL olacak şekilde 2500 mg/kg/gün CCl₄C dozunda oral gavaj ile beslendi ve karaciğer yağlanması oluşturulmaya çalışıldı. Çalışma 4 hafta sürdü. Bilgisayarlı tomografi dansite ölçümleri, her organdan alınan farklı üç 0,5 cm²'lik alanın ortalaması alınarak elde edildi. Veriler patoloji ile karşılaştırıldı. Farelerin karaciğerleri, dalakları, böbrekleri, paravertebral kasları çıkarıldı. Parafin bloklardan elde edilen 5 mikron kalınlığındaki kesitler lama aktarıldı. Karaciğer örneklerindeki yağlanma miktarının ölçümü için interaktif imaj analiz sistemi (Aequitas, Dynamic Data Links) kullanıldı. Her örnek için karaciğer yağlanma oranı belirlendi. Sonuçlar istatistiksel olarak değerlendirildi. **Bulgular:** Karaciğer dansitesindeki 40 Hounsfield ünitelik azalma %30'luk karaciğer yağlanmasına denk gelmektedir. Karaciğer/dalak, karaciğer/böbrek ve karaciğer/kas dansite değerleri ile histomorfometrik karaciğer yağlanması oranı arasında istatistiksel olarak anlamlı bir korelasyon vardı. Karaciğer/dalak bilgisayarlı tomografi dansite oranı 0,32 ve altında olduğunda, transplantasyonda karaciğer yağlanması için kritik değer olan %30 ve üzeri değerden şüphelenilmelidir. **Sonuç:** Bu deneysel çalışmada ortaya koyduğumuz formülle, karaciğer/dalak dansite oranı ölçümü ile total karaciğer yağlanması hesaplanabilir.

Anahtar kelimeler: Karaciğer transplantasyonu, yağlı karaciğer, multidetektör bilgisayarlı tomografi, deneysel hayvan kullanımı, komplikasyonlar

graft and patient survival diminish. Primary graft non-function/failure (PGF) occurs in as many as 9% of transplants. Significant donor liver macrovesicular steatosis (30%-50%) is an important contributing factor (1). PGF is

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unlikely when donor livers with more than 30% steatosis are excluded (2). On the other hand, microvesicular steatosis does not usually adversely affect the clinical course after transplantation (3).

To date, biochemical and imaging data have been insufficient in determining the degree of the hepatosteatosis, and there is no consensus about determining the degree of hepatosteatosis with imaging modalities, especially by computed tomography (CT) (4). Animal models are beneficial for understanding the pathophysiologic mechanisms involved in the ischemic reperfusion damage in fatty livers and also for comparison with histopathology and imaging modalities (5).

The aim of this study was to analyze the degree of hepatosteatosis and CT findings experimentally using the mouse liver to create CT indexes for determining the degree of hepatosteatosis of the donor liver.

MATERIALS and METHODS

This experimental animal study was approved by the Ethics and Animal Care Committee. The institutional and national guide for the care and use of laboratory animals was followed. Forty Swiss Albino mice (mean age: 3 months) weighing 40 grams were obtained from the Experimental Animals Production Center. The mice were given a number by ear punching system for identification of each mouse.

Before the study, whole body CT scans were obtained by Picker MxTwin four-slice multidetector CT (MDCT) device with 90 Kv 150 mAs, 1 mm section thickness spiral examination. Hyoscine butylbromide (0,5 mg/kg) intramuscular was used to reduce gastrointestinal peristalsis. Small abdominal bandages were used to reduce the respiratory motion artifacts. Different reconstruction algorithms were used to reduce artifacts. The window width and center were stated as 150 Hounsfield units (HU) and 50 HU, respectively. Sedation was provided by intraperitoneal administration of 200 mg/kg ketamine and 10 mg/kg xylazine per mouse before imaging. The mice were fed by oral gavage with 0,06 mL carbon tetrachloride CCl₄ plus 0,04 mL corn oil, totally 0,1 mL, in 2500 mg/kg/day CCl₄ dosages. Insulin injectors and angiocath were used for the oral gavage. The mice were fed by oral gavage for four days and with pellet injections of the same dosage for three days each week. A mouse was sacrificed at the end of each week and its liver was evaluated to determine the presence of hepatosteatosis pathologically. In sacrificed animals, fatty changes were seen homogeneously. The first week, three mice died because of pulmonary aspiration of the oral gavage. The

study was continued for four weeks. Thirty-three mice were alive at the end of the study. At the end of the study, whole body CT scans of the mice were reobtained by the same method. Afterwards, mice were sacrificed by ketamine (400 mg/kg dose). The CT density measurements were obtained by taking the average of three different 0,5 cm² areas in every organ. There were homogeneous diffuse fatty changes in the livers of the animals on CT. CT density measurements were performed in the liver segments 8 and 4 approximately 1 cm above the concomitant portal vein. For the evaluation of segment 5, hepatosteatosis measurements were done 1 cm below the concomitant portal vein. The livers, spleens, kidneys, and paravertebral muscles of the mice were removed. After their tissues were fixed in 10% formalin for 24 hours routinely, they were followed up and paraffin blocks were prepared. Five-micron thick slices from the paraffin blocks were mounted to slides. It was attempted to obtain axial sections as CT measurements were performed. Sections were stained with hematoxylin-eosin (HE). A simple morphometric technique was devised that allowed measurement of the degree of steatosis in liver biopsy specimens stained by HE. An interactive image analysis system (Aequitas, Dynamic Data Links) was used to measure the degree of steatosis in liver specimens. A section routinely stained with HE was examined on a microscope linked to a computer. On each image, the area of steatosis was outlined with the drawing facility, and the area was measured. For each specimen, the proportion of the liver with steatosis was determined. These were examined without knowledge of the CT finding.

The percentages of macro, micro and total steatosis were found. Macrovesicular steatosis is with fat droplets (vacuoles) displacing liver cell nuclei to the cell periphery. Microvesicular steatosis is characterized by enlargement of the hepatocytes and flocculent attenuation of the cytoplasm with centrally located nuclei. It is important to recognize the significance of classifying steatosis into macrovesicular and microvesicular because of the different clinical implications (6). Among the same mice, both macrosteatosis and microsteatosis were seen with the same feeds, but this can be explained by the varying metabolisms of each mouse and the metabolic toxic effects of CCl₄.

Results were assessed statistically by Pearson correlation analysis, paired t test, and nonlinear regression analysis for pre- and post-trial parameters using the Statistical Package for the Social Sciences (SPSS) 10,0 for Windows database. In all tests, $p < 0,05$ was found as statistically significant.

RESULTS

Pre-study CT density values of organs, pre-study ratios of the densities of the livers to other organs, post-study CT density values of organs, post-study ratios of the densities of the livers to other organs, post-study liver density reduction amounts, and histopathologic histomorphometric total, micro, and macrosteatosis percentages were calculated (l: liver density, s: spleen density, k: kidney density, m: muscle density, ls: liver/spleen density ratio, lk: liver/kidney density ratio, lm: liver/muscle density ratio, pl: post-study liver density, ps: post-study spleen density, pk: post-study kidney density, pm: post-study muscle density, pls: post-study liver/spleen density ratio, plk: post-study liver/kidney density ratio, plm: post-study liver/muscle density ratio, deltalul: pre- and post-study liver density difference, total: total steatosis percentage, ma: macrosteatosis percentage, micr: microsteatosis percentage). There were fairly significant reductions in the densities of the livers (l: $54,28 \pm 2,8$ HU, pl: $12,43 \pm 15,0$ HU, $p=0,0008$) and significant reductions in the densities of the kidneys (k: $45,24 \pm 3,01$ HU, pk: $44,1 \pm 2,98$ HU, $p=0,045$), but there were no significant reductions in the densities of the spleens (s: $48,37 \pm 3,35$ HU, ps: $47,65 \pm 3,49$ HU, $p=0,062$) and muscles (m: $47,718 \pm 2,25$ HU, pm: $47,715 \pm 2,34$ HU, $p=0,068$) after the study.

The ratios of the densities of the livers to the densities of the other organs in the pre-study were compared to those in the post-study. There were significant reductions in the ratios of liver/spleen, liver/kidney and liver/muscle after the study (ls: $1,1247 \pm 5,708 \times 10^{-2}$, pls: $0,2670 \pm 0,3178$, $p=0,0007$; lk: $1,2032 \pm 7,377 \times 10^{-2}$, plk: $0,2797 \pm 0,3402$, $p=0,0075$; lm: $1,1389 \pm 6,065 \times 10^{-2}$, plm: $0,2683 \pm 0,3226$, $p=0,0078$). Based on the reductions in the densities of the livers, there were statistically significant reductions in the ratios of the densities of the livers to the densities of the other organs, and considering the hepatosteatosi, these reductions seem to give far more valuable information than liver density alterations.

The liver density reduction after the study and the ratios of the liver densities to other organs were compared with histopathologic and histomorphometric total steatosis, macrosteatosis and microsteatosis. There was a statistically fairly significant correlation between liver density reduction (deltalul) and total-macrosteatosis percentage ($r=0,953$, $p=0,0061$ and $r=0,644$, $p=0,0074$) and a significant correlation with microsteatosis ($r=0,530$, $p=0,0086$). While there was a statistically fairly significant correlation between liver/spleen, liver/kidney, liver/muscle ratios and the total-macrosteatosis percentages ($p=0,00083$), there was a fairly significant correlation

between liver/spleen ratios and microsteatosis percentages ($p=0,00077$) and a significant correlation between liver/kidney, liver/muscle ratios and microsteatosis percentages ($p=0,093$). The correlation between the reduction in liver density and total steatosis has been investigated (Figure 1). The formula for "Density reduction as $HU=19,91 \times (\text{hepatosteatosi percentage})^{2,197}$ " gained from the slope formula calculator and total steatosis percentage can be calculated using the density reduction amount. A reduction of 40 HU in liver density is related with 30% hepatosteatosi.

The correlation between the post-study ratios of liver/spleen, liver/kidney, liver/muscle, and total steatosis is illustrated in Figure 2.

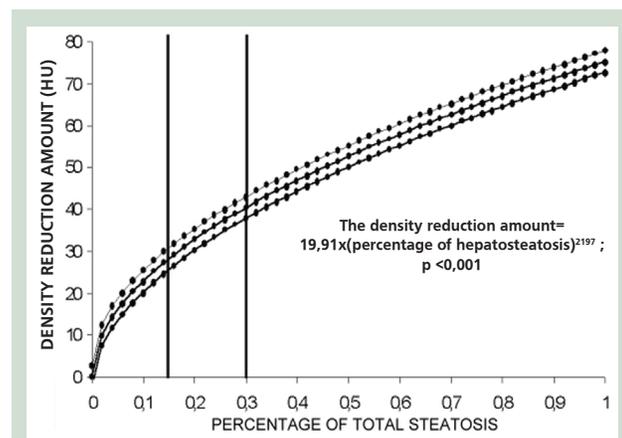


Figure 1. The correlation between the reduction in liver density and total steatosis (While X axis indicates the percentage of total steatosis, Y axis indicates the density reduction amount. The density reduction amount = $19,91 \times (\text{percentage of hepatosteatosi})^{2,197}$; $p < 0,001$).

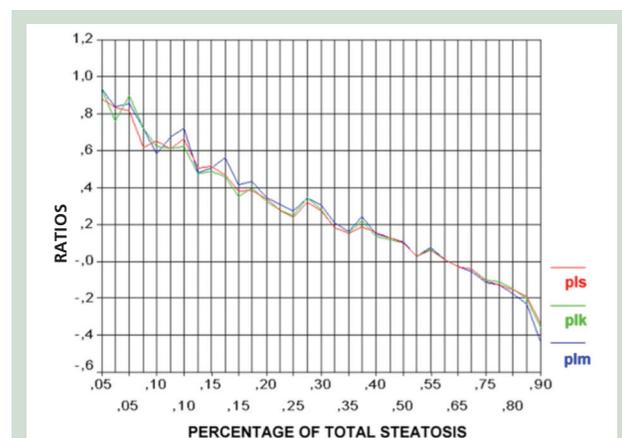


Figure 2. The correlation between total steatosis and post-study liver/spleen, liver/kidney, liver/muscle ratios (pls: post-study liver spleen density ratio, plk: post-study liver kidney density ratio, plm: post-study liver muscle density ratio).

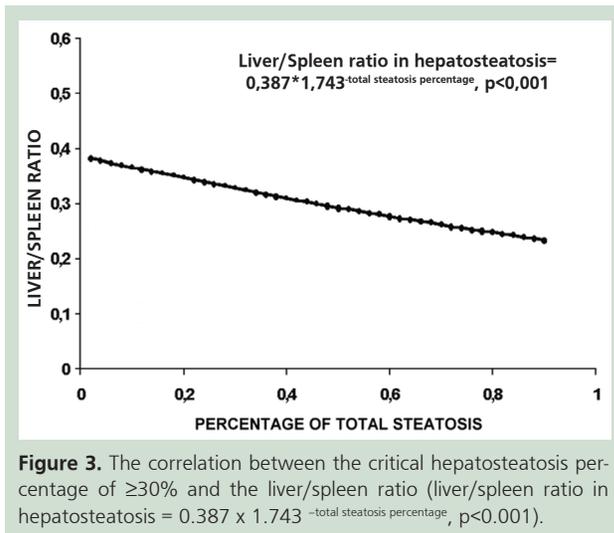


Figure 3. The correlation between the critical hepatosteatosis percentage of $\geq 30\%$ and the liver/spleen ratio (liver/spleen ratio in hepatosteatosis = 0.387×1.743 -total steatosis percentage, $p < 0.001$).

When the liver/spleen ratio is $\leq 0,32$, the critical value for transplantation of $\geq 30\%$ hepatosteatosis must be doubted (Figure 3). Slope formula ("liver/spleen ratio = $0,387 \times 1,743$ -total hepatosteatosis percentage") was obtained with slope formula calculator.

DISCUSSION

According to the results of this study, by using the formula of "Density reduction amount as HU = $19,91 \times$ (hepatosteatosis percentage) $2,197$ ", total steatosis percentage can be calculated using the amount of density reduction. According to this formula, a reduction in liver density of 40 HU indicates 30% hepatosteatosis. Since hepatosteatosis of $\geq 30\%$ is the limit indicating consideration of the risks for liver transplantation, it can be admitted as the critical value. However, it is impossible to know the density alteration in a fatty donor liver. If the normal liver density values are determined according to age, sex and other demographic characteristics in the clinical application, the percentage of hepatosteatosis in the density alteration axis will be calculated comfortably.

There was a statistically significant correlation between the liver/spleen ratio and the histomorphometric hepatosteatosis percentage. In addition to the liver/spleen ratio, there was a statistically significant correlation between the liver/kidney and liver/muscle ratios and the histomorphometric hepatosteatosis percentage.

To our knowledge, this is the first demonstration in the literature of the correlation between the liver/spleen ratio, liver/kidney ratio, liver/muscle ratio and the histomorphometric hepatosteatosis percentage and of the formulation of the correlation between the liver/spleen ratio and the histomorphometric hepatosteatosis percentage.

According to this, total hepatosteatosis can be calculated using the formula "liver/spleen ratio = $0,387 \times 1,743$ -total hepatosteatosis percentage". When the liver/spleen ratio is $\leq 0,32$, then the critical value for transplantation of $\geq 30\%$ hepatosteatosis must be doubted. In the presence of pathologies other than fatty infiltration that will cause a decrease in spleen attenuation, we have come to the conclusion that liver/muscle or liver/kidney ratios are usable for the calculation of the total hepatosteatosis percentages.

If there are no alternatives in treatment, liver transplantation is an effective therapeutic modality in irreversible acute and chronic liver disease. The presence and degree of hepatosteatosis in the liver transplantation must be determined carefully because these concepts are important for graft and recipient survival and donor liver sufficiency.

There are large series focusing on the importance of hepatosteatosis in liver graft damage and dysfunction (7-10). There are some reports indicating that even mild hepatosteatosis may affect patient and graft survival (8-11).

Graft survival decrease due to hepatosteatosis is seen more often in the early posttransplantation period. In contrast to macrovesicular steatosis, microvesicular steatosis causes less graft damage and produces graft survival rates similar to the graft without steatosis (12,13).

The effects of steatosis on graft and patient survival are more certain in the liver recipient in a critical status. There is consensus about using mildly and not severely steatotic grafts, but discussions are ongoing about using intermediate steatotic grafts (14-16).

Most of the steatotic donor livers are composed of macrovesicular fat globules. In the study of Zamboni et al. (12), it was found that there was a correlation between macrovesicular steatosis $>25\%$ and short patient survival. They defined this kind of liver as a marginal graft. In a study by Salizzoni et al. (11), there was a correlation between macrovesicular steatosis occurring in as many as 15% of the hepatocytes and short patient-graft survival, and these grafts were defined as marginal grafts.

Although microvesicular steatosis does not usually adversely affect the clinical course after transplantation (3,17), there are some researches that indicate high-grade microsteatosis usually coexists with macrosteatosis, and the presence of high-grade microsteatosis was significantly associated with delayed hepatic function (18,19).

The staging of the hepatosteatosis is made by using the semi-qualitative determination methods of the fat amount in the liver as mild, intermediate or severe. In most of the series, hepatosteatosis of $<30\%$ is classified

as mild, 30-60% as intermediate and >60% as severe (15). Because of the effects of hepatosteatosis on liver regeneration, liver resection may cause problems in living liver donors and recipients. After the liver resection, there is an increasing risk of mortality among hepatosteatotic patients compared to normal patients (14% versus 2%) (20). The prevalence of hepatosteatosis in cadaveric liver donors varies between 13% and 28%, and when using the most sensitive histologic techniques, this rate approaches 50% (21). Severe and moderate degrees of total steatosis were seen in 0,6% and 10,8% of subjects, respectively, in living liver donors without evidence of fatty liver on ultrasonography (USG) (22).

The simplest and most common method in determining the existence and degree of hepatosteatosis is the body mass index (BMI) obtained by the division of body weight in kilograms by height in meters squared (23-25). However, BMI above a certain value (e.g.: BMI >28) (23) predicts the need for biopsy. In this regard, BMI could be a guide before the liver biopsy.

During the laparotomy, it is possible to determine severe hepatosteatosis with the naked eye. The naked eye inspection with respect to severe, intermediate and mild hepatosteatosis has positive predictive values of 71%, 46% and 17%, respectively (26). This method cannot provide a quantitative assessment of hepatosteatosis and is based on the surgeon's judgment.

Although the histologic examination of the liver for hepatosteatosis is accepted as the gold standard, the slice thickness and the staining techniques have effects on the determination of the presence and degree of hepatosteatosis (27). In determining hepatosteatosis, HE is more commonly used among the staining techniques (15). Histologic examinations require invasive procedures and bring into play the possible complications. When there is heterogeneity in the distribution of the hepatosteatosis, a single biopsy sample is insufficient in the assessment (28). The other disadvantage of biopsy is the time requirement. In the study of Joy et al. (29), it was stated that biopsy was the gold standard method, but noninvasive techniques were required.

Even the liver imaging methods have limited roles in the assessment of cadaveric liver donors. They have much more importance in the determination of the presence and degree of hepatosteatosis in living liver donors. USG is cheaper than CT and magnetic resonance imaging (MRI), but its specificity is lower. MRI and magnetic resonance spectroscopy (MRS) are good alternatives in the assessment of hepatosteatosis, and the studies are ongoing (30,31). MRI and MRS require much more examina-

tion time than USG and CT. The costs of MRI and MRS are higher than that of CT and are less available. These are the disadvantages of MRI and MRS.

Noncontrast CT is the most accurate and effective imaging technique in determining the presence and characterization of hepatosteatosis (32). In noncontrast CT, the liver attenuation values are higher than those of the spleen. If these values are reversed or the liver-spleen attenuation difference is above -8 or -10, it can be considered diagnostic for hepatosteatosis. The low attenuation values in hepatosteatosis are based on the triglyceride and cholesterol accumulation in hepatocytes.

Although the qualitative determination of hepatosteatosis is important, its quantitative assessment is necessary in living liver donors and in the treatment and follow-up of patients with hepatosteatosis, nonalcoholic fatty liver or steatohepatitis. The studies related with the assessment of fatty liver have focused on the quantitative determination of the fat.

Ricci et al. (33) stated that the fat amount in the human liver could be measured using a noninvasive quantitative method by performing CT calibration with the test objects including the fat equivalent materials in increasing amounts (0%, 20%, 30%, 40%) and the liver tissue equivalent material. They emphasized that the CT density measurements were correlated with histomorphometric analysis. According to Ricci et al. (33), absolute HU values and liver/spleen ratios in classical CT methods could be influenced by the volume-related fluctuations, noise and liver and spleen sizes. Thus, calibrated CT was used to avoid these influences (33). In a similar study by Cheng et al. (34), they stated that the calibrated CT using the test tubes would determine hepatosteatosis accurately in a noninvasive quantitative method, and that calibrated CT was the potential parameter in the assessment of living donors. The problems of the standardization and homogenization of the tissue equivalent and fat equivalent materials and the need for the technique test materials are the unfavorable aspects of this method. In our study, we used spiral CT and reconstructed images to avoid the influences of the volume-related fluctuations, noise and liver and spleen sizes. In addition to the liver/spleen ratio, by using liver/kidney and liver/muscle ratios, we aimed to minimize the reported side effects and determine the percentage of hepatosteatosis accurately.

Dual energy CT is another method in the determination and quantitation of hepatosteatosis (35). Dual energy CT requires two examinations and increases the cost. The points that should be discussed are which energy levels are useful and the relationship between the different

energy levels and the volume measurements in steatotic livers.

Performing this study with “small” laboratory animals is a limitation of this study. However, the Ethics and Animal Care Committee gave permission to perform this study on small laboratory animals. Practical application of the formulas and data on human subjects may necessitate

more clinical researches.

We conclude that with the measurement of the liver/spleen ratio, the total hepatosteatosis percentage can be calculated according to the formula, which we introduced herein, based on our experimental study.

“The authors declare no conflicts of interest.”

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