

The impact of ultrastructural tail abnormalities on sperm motility among infertile men

Original Article

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ABSTRACT

Background: Asthenozoospermia is a status of low or absent sperm motility in fresh ejaculate. Absolute asthenozoospermia, i.e., 100% immotile sperms, is reported in one of 5000 men. Many factors are involved in impaired sperm motility and in turn male factor infertility. The ultrastructure defects of sperm tail are considered the most important factors that cause severe asthenozoospermia.

Aim: To evaluate the ultrastructure abnormalities of the sperm tail in infertile men with severe asthenozoospermia.

Patients and Methods: This study was conducted on 22 asthenozoospermic (total motility < 5%) infertile patients and 10 matched fertile controls. Sperm motility was evaluated by Computer-Aided Semen Analysis (CASA). Ultrastructural defects of the sperm tail were evaluated using transmission electron microscope (TEM).

Results: Sperm tail defects were evident in 100% of the examined cases in this order of frequency : microtubular disturbance (91%), mitochondrial anomalies (45%), and fibrous sheath dysplasia (9%). Most of the cases showed more than one anomaly. Patients with mitochondria anomalies had the least total motility (1.93 ± 1.73), while patients with microtubular disturbance had the highest total motility (3.02 ± 0.53). Moreover, patients with complex anomalies revealed the least total motility (1.5%).

Conclusion: Transmission electron microscope is a vital step that should be performed in infertile men with severe asthenozoospermia who are resistant to medical treatment.

Key Words: Asthenozoospermia, computer-aided semen analysis, infertility, transmission electron microscope.

Received: 17 September 2023, **Accepted:** 17 October 2023

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ISSN: 2090-6048, 2023

INTRODUCTION

Decreased or absent sperm motility (i.e., asthenozoospermia) is considered one of the main causes of malefactor infertility^[1]. It could be attributed to genital infections, maturation abnormalities in the epididymis, abnormalities in the seminal plasma, and intrinsic or metabolic defects in the flagellar axonemes^[2].

Among 5000 individuals, there is one male with 100% immotile sperms, a condition called absolute asthenozoospermia^[1]. Such condition is mostly attributed to ultrastructure abnormalities of the sperm tails, which result from genetic defects in the process of spermiogenesis^[3,4].

Transmission electron microscope (TEM) is demonstrated for characterizing and measuring the thermodynamic, electric, and mechanical properties of individual nanostructures and organelles^[5]. It is a gold standard technique in the evaluation of the integrity of the sperm-specialized ultrastructure^[6]. It could study the defects

of different sperm components that influence fertilizing potential^[7,8].

The midpiece area of the human sperm is referred to as a coiled helix of 12–13 turns of mitochondria enveloping the axoneme and the outer dense fibers (ODF) ending with a ring-shaped structure, the annulus^[9]. Dysfunctions of the human mitochondrial sheath as well as mitochondrial membrane integrity represent important causes of asthenozoospermia^[10].

The axoneme, the propulsive engine of spermatozoa, is composed of nine doublet microtubules surrounding two central, singlet microtubules, and hence the term, the '9+2' axoneme. These microtubules are surrounded by fibrous sheath. Axonemal anomalies are related to the number and position of microtubules^[8,11]. The structural defects, which include the number of ODF, and the organizations of fibers, are associated with motility problems^[12].

The aim of this study is to evaluate the ultrastructure

abnormalities of the sperm tail in patients with severe asthenozoospermia and their contribution to infertility.

PATIENTS AND METHODS

This study was conducted on 22 severe asthenozoospermic (total motility < 5%) infertile men attending the Andrology Outpatient Clinic, and 10 matched fertile controls. Patients with increased seminal viscosity, pus cells, urogenital infection, endocrine disease, psychiatric disorders, and patients receiving chemotherapy or radiotherapy were excluded from the study. Patients were subjected to complete history-taking and general and local examination. Written consents were signed by all participants. The study was approved by the university's Ethical Research Committee.

CASA procedure

Semen samples were collected by masturbation into sterile plastic jars after 3–5 days of sexual abstinence. After liquefaction, assessment of sperm count($\times 10$), total and progressive motility percentage, as well as morphology was performed according to WHO guidelines (WHO, 2010) using Spermyser software (v. 1.2, Miralab, Egypt).

TEM procedure

Fixation and preparation of seminal fluid for TEM was performed based on the instruction manual to obtain the embedded blocks. Semithin sections were obtained using L K B ultramicrotome (Germany). These sections were prepared for detection of tissue orientation and photographed by sc30 Olympus camera (Japan).

Ultrathin sections were done using Leica AG ultramicrotome (Germany). The sections were later examined by JEM 100 CXII electron microscope at 80

KV (Japan) and photographed by CCD digital camera (Model XR-41, Japan).

RESULTS

Demographic and clinical data

This prospective, controlled cross-sectional study was conducted on 22 asthenozoospermic 1ry infertile patients and 10 matched healthy controls. The age of the studied cases ranged from 21 to 49 years (mean \pm SD = 31.9 ± 1.92 , median = 31). The duration of infertility ranged from 2 to 11 years (mean \pm SD of 4 ± 0.55 , median = 4).

CASA results

Total sperm count ranged from 13 to 193 million/ ejaculate among patients (mean \pm SD = 76.2 ± 22.5 , median = 57), and 65 to 250 million/ ejaculate among healthy controls (mean \pm SD = 166 ± 20.5 , median = 57), while sperm concentration ranged from 3.3 to 92 million/ ml among patients (mean \pm SD = 28 ± 8.7 , median = 14), and 53 to 117 million/ ml among controls (mean \pm SD = 68 ± 16.4 , median = 57). Total sperm motility ranged from 0 to 4.82 among patients (mean \pm SD = 2.8 ± 4.42 , median = 3.55), and 50 to 82 among controls (mean \pm SD = 60 ± 8 , median = 58). Morphology index ranged from 0 to 20 among patients (mean \pm SD = 6.42 ± 1.73 , median = 5), and 4 to 23 among controls (mean \pm SD = 13.55 ± 4.73 , median = 11.5) (Table 1).

TEM results

Sperm tail defects were evident in all patients (100%): one anomaly in 14 (63%) patients, two anomalies in six (27.2%) patients, and three anomalies in two (9.09%) patients. Anomalies were classified in order of frequency into three groups: microtubular disturbance (20 patients, 91%), mitochondrial anomalies (10 patients, 45%), and fibrous sheath dysplasia (two patients, 9.09%) (Table 2).

Table 1: Semen parameters of study participants

Semen parameter	Case (n=22)	Control (n=10)
Sperm count		
Mean \pm SD	76.2 ± 22.5	166 ± 20.5
Median	57	57
Sperm motility		
Mean \pm SD	2.84 ± 0.42	60 ± 8
Median	3.55	58
Morphology index		
Mean \pm SD	6.42 ± 1.73	13.55 ± 4.73
Median	5	11.5

Table 2: Sperm tail defects and their corresponding number of patients

Anomaly	Sperm tail defect	Number of patients
Microtubular	Reduced number of doublets	22
	Disturbed arrangement	22
	Absent centriole	2
Mitochondrial	Reduced number of gyri	6
	Reduced density of gyri	4
	Disturbed arrangement of gyri	6
Fibrous sheath	Dysplastic irregular fibrous sheath	2

Microtubular anomalies included reduced number of nine doublets of microtubule with an average of 3–6 doublets (22 patients, 100%), disturbed arrangement of microtubules (22 patients, 100%), and absent 2 centrioles (two patients, 9.09%). Mitochondrial anomalies were divided into those related to the number of mitochondrial gyri, which is normally 11–13, the arrangement of mitochondrial helix, and the density. Six (27.2%) patients showed reduced number of mitochondrial gyri from 6 to 9. Six (27.2%) patients showed disturbed arrangement of mitochondrial helix, and four (18.2%) patients showed reduced density (i.e., pale mitochondria). Dysplastic irregular fibrous sheath was associated with mitochondrial and microtubular anomalies in only two patients (Figs. 1, 2).

Patients with mitochondria anomalies had the least

total motility that ranged from 0 to 4.61% (mean \pm SD = 1.93 ± 1.73 , median = 1.61), while patients with microtubular disturbance had the highest total motility that ranged from 1.11 to 4.8% (mean \pm SD = 3.02 ± 0.53 , median = 2.78). Patients with dysplastic fibrous sheath had a total motility from 1.1 to 4% (mean \pm SD = 2.32 ± 0.83 , median = 1.68).

Relating the total number of sperm tail anomalies to asthenozoospermia revealed that patients who had complex abnormalities, i.e., 6 defects, had the least total motility (1.5%), while patients with 2 anomalies had total motility ranged from 1.1 to 4.6% (mean \pm SD = 2.9 ± 0.4 , median = 3.25), and patients with single anomaly had total motility ranged from 0 to 4.6% (mean \pm SD = 3.5 ± 0.78 , median = 3.25).

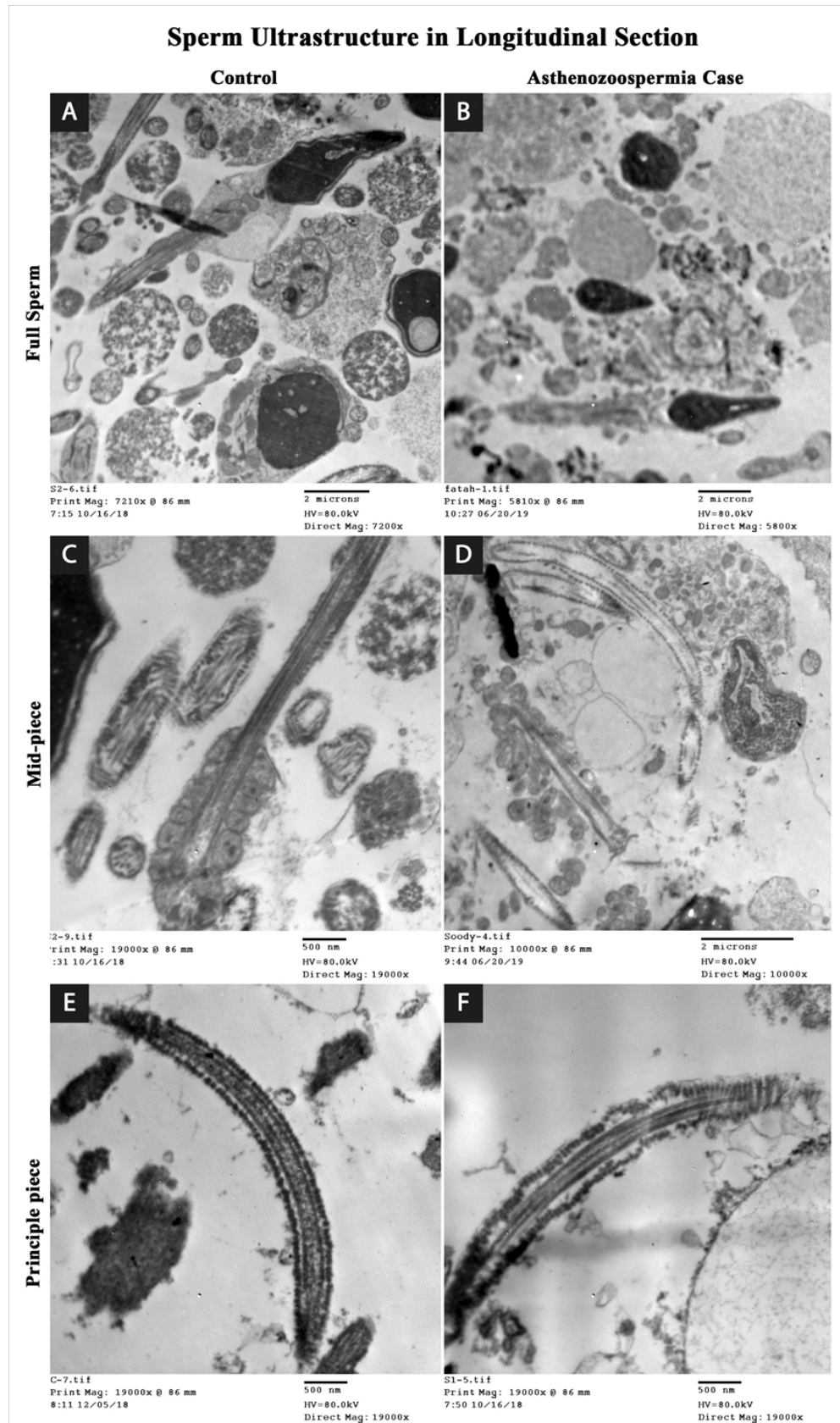


Fig. 1: Longitudinal section of the abnormal ultrastructure of full sperm showing reduced size and density of mitochondria (i.e., pale) (B) compared with the normal full sperm (A). The abnormal midpiece shows reduced size and density of mitochondria gyri (D) compared with the normal midpiece with normal size, number, and density of mitochondrial gyri (C). Also, the abnormal principle piece shows marked disturbed arrangement of the fibrous sheath (F) compared with normal principle piece with normal arrangement of the fibrous sheath (E).

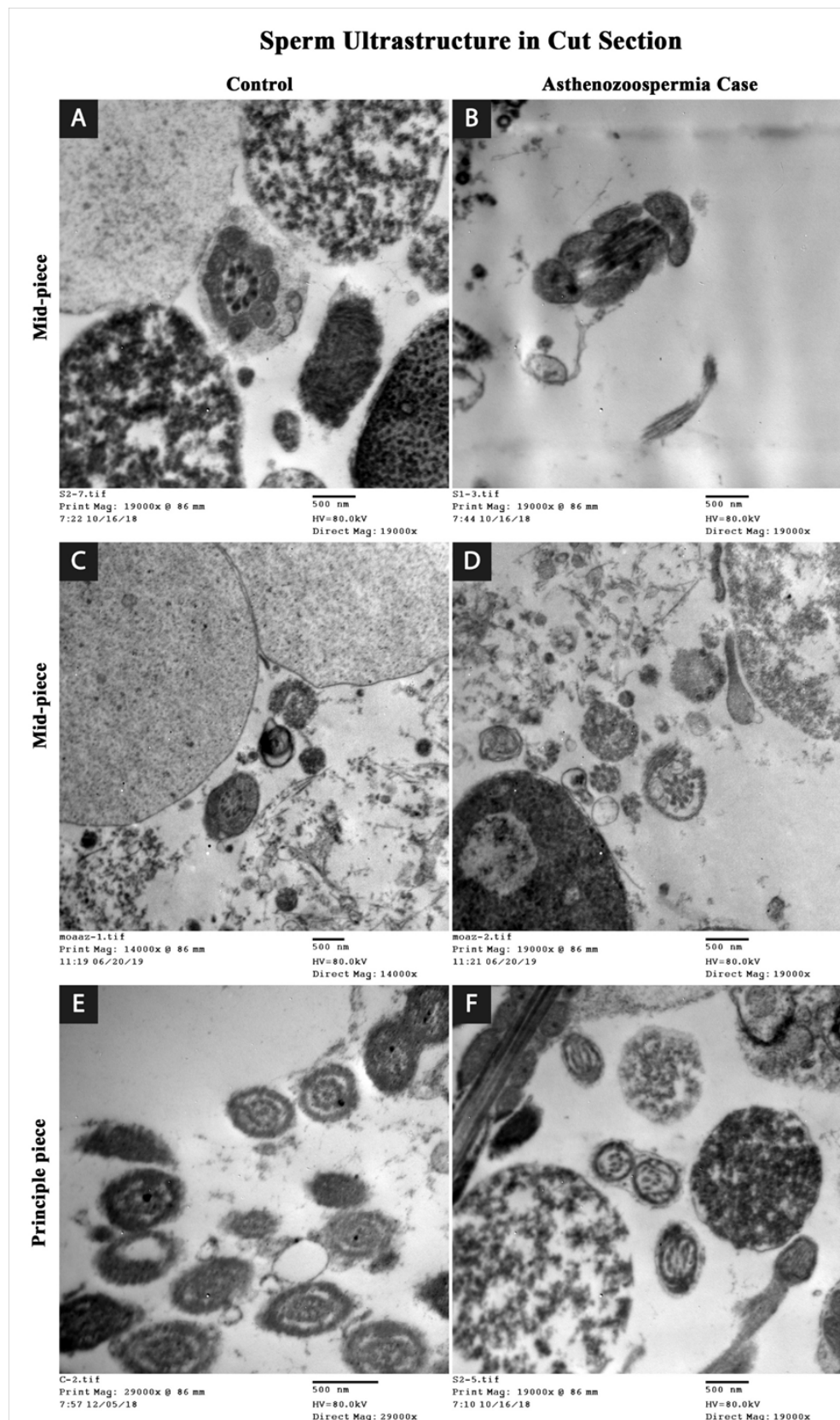


Fig. 2: Cut section of the abnormal ultrastructure of sperm at the level of midpiece showing reduced density and number of mitochondrial gyri (B) as well as reduced density of the mitochondrial ring (D) compared with normal midpiece with normal number, and density of mitochondrial gyri (A) as well as normal density of mitochondrial ring (C). Also, the abnormal principle piece shows disturbed number and arrangement of microtubules (F) compared with normal principle piece with normal number and arrangement of microtubules (E).

DISCUSSION

Sperm motility is crucial for sperm migration to the Fallopian tubes, penetration of the cumulus oophorus, and finally fertilization. Therefore, increased probability of natural conception is linked to normal sperm motility^[13]. Precise evaluation of sperm motility depends mainly on flagellar beating and spatial displacement of the sperm. This is not achieved by CASA that evaluates motility through tracking the sperm head only^[14].

The powerful resolution and magnification of TEM have overcome the limitations of light microscopy. TEM enables examination of the internal sperm structures and the study of its different organelles^[15]. It avoids the empirical analysis of sperm anomalies and allows a deeper understanding of the mechanisms beyond failed reproduction, thus allowing the proper choice of therapy^[16].

This study was conducted on 11 patients with severe asthenozoospermia (total motility < 5%) and 4 matched healthy controls. Using TEM, the ultrastructural defects of the sperm tails and their relation to sperm motility were evaluated. Three main anomalies were observed in the examined sperm tails that involved the microtubules, the mitochondria, and the fibrous sheath. Although patients with microtubular anomalies were the commonest, they did not have the least total motility as compared with other cases. Serres and colleagues had showed that the sperm flagellar structures, including the doublets of microtubules, dynein arms, radial spokes, and centrioles, are active motile elements essential for motility^[17]. Their ultrastructural defects are associated with asthenozoospermia and infertility^[18].

Patients with flagellar defects should be examined and investigated for other disorders associated with ciliopathies such as primary ciliary dyskinesia and situs inversus. Clearly, this indicates a highly complex set of interacting genetic pathways controlling the assembly of cilia and flagella, their motile functions, and their signaling functions^[19].

In this study, patients with mitochondrial anomalies (i.e., disturbed density, arrangement, and number of mitochondrial gyri) had the least total and progressive motility although they are less common than flagellar defects. Mitochondrial anomalies were first described by Gopalkrishnan and colleagues and they were associated with severe asthenozoospermia^[20]. Also, Wilton and colleagues and Mundy and colleagues had described abnormalities of mitochondrial organization, including a shorter midpiece with fewer mitochondrial gyri, total absence of mitochondria from the midpiece, lack of the mid-piece segment, as well as bad assembly or clustering of mitochondria^[2,21]. Piasecka and colleagues had evaluated different mitochondrial changes such as mitochondrial membrane potential^[22].

The authors pointed out that in some cases of asthenozoospermia, sperm mitochondria can be functionally active with a high mitochondrial membrane potential. Therefore, the low sperm motility does not necessarily result from energetic disturbances, but it may be associated with structural deformations. Ferreux and colleagues considered that mid-piece defects causing severe asthenozoospermia and lower fertilizing potential might imply negative prognostic factors in ART. Patients with mitochondrial defects that were subjected to ICSI showed 50% conception rate, but unfortunately it was followed by miscarriage at the end of the first trimester^[23].

The third major observed anomaly was hyperplastic and disorganized fibrous sheath. DFS was the least commonly encountered anomaly that was seen in only one case. This patient had total motility better than patients with mitochondrial structural defects but less than patients with microtubular defects. Escalier and David (1984) observed that the fibrous sheath anomaly was a very rare human sperm tail anomaly^[12]. According to Chemes and colleagues (1998), the main TEM features of spermatozoa with DFS are marked hypertrophy and randomly arranged fibrous sheath constituents^[24]. Moreover, Sironen and colleagues had described a new variant of the immotile cilia syndrome in which DFS was associated with the classical dynein arm deficiency in cilia and spermatozoa^[25]. In our work, DFS was associated with complete distorted 9+2 arrangement of microtubules in both mid- and principal pieces. Baccetti and colleagues (2005) showed that ICSI provides a suitable solution for patients suffering from irreversible sperm defects such as DFS, and the prognosis of ICSI in such patients was better than those with mitochondrial defects^[26].

TEM enables examination of the internal sperm structures and the study of its different organelles. It is the best method to study the etiology of teratozoospermia, including defects of sperm head, tail, and distorted arrangement and structure of the sperm organelles that influence fertilizing potential^[27]. Recently, progress in CASA technology is resolving many of image analysis problems, based on availability of higher-resolution digitizers and greater computing power combined with software features such as automated and/or interactive illumination control, advanced motion-filtering, drift-filtering, and tail-tracking^[28].

We recommend doing TEM examination on a larger number of infertile males to propose how frequent the EM defects are present, and we recommend also following up the results of ART in those patients to see which technique gives better results in different defects.

CONCLUSION

Transmission electron microscope is a vital step that should be performed in infertile men with severe asthenozoospermia who are resistant to medical treatment.

CONFLICT OF INTEREST

There are no conflict of interest.

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