Ultrasound Vascular Elastography as a Tool for Assessing Atherosclerotic Plaques – A Systematic Literature Review

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Abstract
Atherosclerosis is a widespread disease that accounts for nearly 3-quarters of deaths due to cardiovascular disease. Ultrasound elastography might be able to reliably identify characteristics associated with vulnerable plaques. There is a need for the evaluation of elastography and its ability to distinguish between vulnerable and stable plaques. The aim of this paper is to provide an overview of the literature on vascular elastography. A systematic search of the available literature for studies using elastography for assessing atherosclerotic plaques was conducted using the MEDLINE, Embase, Cochrane Library and Web of Science databases. A standardized template was used to extract relevant data following the PRISMA 2009 checklist. 20 articles were included in this paper. The studies were heterogeneous. All studies reported that elastography was a feasible technique and provided additional information compared to B-mode ultrasound alone. Most studies reported higher strain values for vulnerable plaques. Ultrasound elastography has potential as a clinical tool in the assessment of atherosclerotic plaques. Elastography is able to distinguish between different plaque types, but there is considerable methodological variation between studies. There is a need for larger studies in a clinical setting to determine the full potential of elastography.

Introduction
Atherosclerosis is a slowly progressive disease that accounts for nearly 3-quarters of deaths due to cardiovascular disease. Cardiovascular disease is the leading cause of mortality worldwide [1]. Despite the clinical significance of atherosclerotic plaques, it is difficult to assess the risk of plaque rupture and thrombosis with current imaging modalities [2]. Recently plaque morphology, histology and mechanical properties have gained interest as potential important factors for discerning vulnerable from stable plaques. Evidence in the published literature suggests that ischemic events are more frequent in patients with plaques containing a large lipid core than stiffer calcified and fibrous plaques [3,4]. Ultrasound is commonly used for both the evaluation of plaque morphology and the evaluation of the hemodynamic consequences of plaques in blood vessels. Ultrasound elastography evaluates tissue stiffness. Several ultrasound elastography techniques are available [5]. 2 techniques, strain and shear wave elastography, have been applied in the assessment of vascular plaques [6,7]. In strain elastography mechanical stress is applied manually by compressing the skin with the transducer or by physiological motion, i.e., arterial pulsation. The tissue strain is measured relative to the surrounding tissue and translated into a color-coded map, which may be shown as an overlay on the B-mode image. The method is qualitative, but semi-quantitative measurements may be made where the strain in a region of interest is calculated relative to the surrounding tissue. Shear wave elastography is a quantitative method in which tissue displacement is caused by an ultrasound push pulse. Tissue displacement is measured by measuring the speed of shear waves occurring perpendicularly to the ultrasound push pulse [6]. Strain elastography is well evaluated in breast tumors, thyroid nodules and lymph nodes, whereas shear wave elastography is well evaluated in liver fibrosis assessment and breast tumors [8]. Vascular elastography may be performed endovascularly or noninvasively. Elastography images or measurements may be used to differentiate between vulnerable and stable plaques. Elastography could potentially play an important role in identifying vulnerable...
plagues and help gain insight into the pathophysiology of acute coronary syndrome or stroke and identify high-risk patients. The aim of this paper was to provide a systematic overview of the literature on the use of vascular ultrasound elastography.

Methods

Search strategy and selection
A literature search was performed in MEDLINE, EMBASE, Web of Science and the Cochrane Library databases. We use MEDLINE here as an example of the systematic searches. In Medline the following search terms were applied: the MeSH terms used were “elastility imaging techniques” AND “plaque, atherosclerotic”. To ensure the inclusion of studies not yet indexed with MeSH terms, a free text search was performed using the terms “acoustic radiation force imaging” OR “real-time tissue elastography” OR “supersonic” OR “Fibroscan” OR “elasticity imaging techniques” AND “atherosclerosis” AND “plaque”. 2 authors reviewed all titles and abstracts (B. Mahmood and C. Ewertsen). Eligible papers were read by 2 of 3 authors (B. Mahmood, J. Carlsen and C. Ewertsen). Only original research articles in English concerning ultrasound elastography of atherosclerotic plaques in humans, animals or phantoms were included. Consensus was obtained through discussion. Furthermore, all reference lists of included articles were hand-searched for further references. The following data were extracted from the included manuscripts: a) type of study, b) elastography method, c) study population, d) number of subjects, e) method of comparison, f) artery examined, g) quantification method, h) outcome of study and i) any additional relevant information about the studies. The following exclusion criteria were used: editorials, reviews, case reports investigating 5 subjects or less, letters to the editor, conference abstracts and articles on mathematical optimization of elastography. The PRISMA 2009 checklist was used as a guideline for reporting the findings for each article [9]. Before the search process was initiated, the aim of the review was agreed upon. The searches were performed on April 9, 2016.

Results

Selection of studies and overview
The initial search yielded 219 citations. Based on the title, 182 articles were excluded (including duplicates) and another 9 articles were excluded after review of the abstract. Fig. 1 shows a flowchart of the systematic review process. 28 articles were retrieved for full text reading. 20 studies were eligible and therefore included in the final analysis. 4 studies investigated elastography in either humans or animals in addition to phantom studies and are therefore presented in more than one subgroup in Table 1. A predefined standardized template for data extraction was employed for all studies. The elastography methods employed in all of the included studies are presented and described in Table 2. In 12 of the studies data was acquired from human subjects. 4 studies included data from animals: 3 were performed on pigs and one study was conducted on rabbits. The remaining 4 articles described data from either phantoms only or a combination of phantoms and humans or animals. No cut-off values for measured strain in plaques were described in the included studies to differentiate between vulnerable and stable plaques.

Noninvasive elastography
To evaluate plaque stiffness, a noninvasive approach was employed in 10 of the included articles in human subjects [10–19]. 8 studies investigated elastography in humans only and 2 studies investigated elastography in both humans and phantoms [13,19]. 8 studies used strain elastography and 2 studies used shear wave elastography [12,14] to assess plaques stiffness. In 8 studies strain or shear wave values were obtained from atherosclerotic plaques in the carotid, whereas 2 studies assessed strain values from the femoral and/or popliteal artery [13,18]. All studies reported that noninvasive elastography was able to distinguish between different types of plaques, however with different methods of reference. [6] studies describe a positive correlation between tissue strain and plaque vulnerability [12,14,15,17–19]. Only one study found that mean strain values were significantly lower in vulnerable plaques implying that vulnerable plaques were less stiff than non-vulnerable plaques [11]. This study estimated mean strain values for the entire plaques while other studies evaluated plaques based on smaller regions of interest and maximal measured strain values. 2 strain elastography studies and one shear wave elastography study compared elastography findings with histology and concluded that elastography could differentiate between plaques with fibrous tissue, lipid core, intra-plaque hemorrhage and foam cells [12,15,19]. 2 studies investigated the relationship between the patient’s neurological status and plaque strain and found that patients with focal neurological symptoms had plaques with higher strain values, which represented soft plaques [17,20]. The above mentioned studies described plaques with 2 parameters: high strain spots and/or average strain values for the entire plaques. Only plaques with focal high strain spot values were associated with diminished cognitive function. 2 studies compared their strain investigation with B-mode images and found that strain values provided additional information compared to B-mode only [10,13].

Endovascular elastography
In 2 studies endovascular elastography, a method based on strain elastography, was used to evaluate atherosclerotic plaques in coronary arteries in-vivo in humans [21–23]. Endovascular elastography was able to distinguish between plaques and their composition in both studies, although these studies were quite heterogeneous. In the first study the outcome was compared to histology [21], and in the second it was compared to B-mode images [23]. Only strain elastography has been used so far in endovascular elastography studies. Lower mean strain values were reported for vulnerable plaques compared to stable plaques. No difference in mean strain values was reported between normal artery intima and calcified plaques. One study evaluated inflammation measured by histologic presence of macrophages and reported higher strain values for plaques with inflammation compared with plaques without inflammation [21]. In one of the studies lower strain values were reported after atherectomy was performed as an intervention [21].

Elastography in animals
Elastography was evaluated in animals in 5 studies [5,22,24–26]. As presented in Table 1, 3 studies investigated vascular elastography in animals only, while 2 studies assessed elastography in both animals and phantoms [25,26], the latter 2 are therefore presented twice in Table 1. All 5 studies used an invasive approach to assess strain values in animals. 4 studies
were performed in pigs and one in rabbits [24]. One study investigated plaques in the iliac and/or femoral artery [22], while the other studies were performed on the carotid artery; abdominal aorta; renal and coronary arteries, respectively [5, 24–26]. Histology was the method of comparison in all of the animal studies. The histological parameters evaluated for plaque vulnerability differed between the 4 studies, but all of them reported higher strain values in vulnerable plaques.

### Elastography in phantoms and excised vessels

7 studies were performed in phantoms or in excised vessels from either humans or animals [13, 14, 19, 25–28]. 2 of the 7 studies had an additional in-vivo data collection from animals [25, 26], and similarly 2 phantom studies investigated elastography in humans in-vivo [13, 19]. Most studies performed in phantoms and excised vessels were performed to evaluate new mathematical models for calculating strain. These studies were heterogeneous, but all found that high strain values were associated with vulnerable plaques. Excised human vessels were examined in 3 studies, where strain values were compared to histology [19, 27, 28]. 2 of these studies described an association between vulnerable plaques and high strain values [27, 28], and one study reported that noninvasive elastography was feasible on the carotid arteries [19]. In 2 studies strain methods were evaluated in phantoms and followed by validation in pigs [25, 26]. Both studies found that elevated strain was correlated to histologically proven vulnerable plaques.

### Discussion

All 20 studies included in this review reported that ultrasound elastography was able to distinguish between different types of heterogeneous atherosclerotic plaques and that additional information was obtained compared to B-mode ultrasound only. Both endovascular elastography and noninvasive elastography methods were used. Both in-vivo and ex-vivo endovascular elastography studies showed that increased strain is associated with histologically vulnerable plaques [21, 23, 27]. No cut-off values for distinguishing between vulnerable and stable atherosclerotic plaques were suggested for future implementation. To our knowledge this is the first systematic review to evaluate the available literature on ultrasound elastography studies assessing atherosclerotic plaques.

Noninvasive methods for evaluating atherosclerotic stenosis and the risk for stroke include duplex ultrasonography which is recommended as the first choice by North American guidelines [29–31]. The method uses flow velocity-based estimation for measuring the severity of stenosis, but does not indicate whether a particular plaque may be prone to rupture, and does not directly measure the diameter of the artery. Among the pitfalls...
## Table 1  Characteristics of included studies.

<table>
<thead>
<tr>
<th>Author, year and country</th>
<th>Type of study; study population (no. of subjects)</th>
<th>Elastography method; quantification</th>
<th>Method of comparison</th>
<th>Artery</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dahl et al., 2009, USA [10]</td>
<td>Noninvasive; human (23)</td>
<td>ARFI * ; quantitative</td>
<td>B-mode</td>
<td>Carotid</td>
<td>ARFI was able to discriminate between different plaque areas in heterogeneous plaques.</td>
</tr>
<tr>
<td>Naim et al., 2013, Canada [11]</td>
<td>Noninvasive; human (31)</td>
<td>Strain elastography; semi-quantitative</td>
<td>MRI</td>
<td>Carotid</td>
<td>The strain was significantly lower in plaques containing a lipid core compared with those without.</td>
</tr>
<tr>
<td>Garrard et al., 2015, UK [12]</td>
<td>Noninvasive; human (25)</td>
<td>Shear wave; quantitative</td>
<td>Histology</td>
<td>Carotid</td>
<td>The mean Young Modulus of vulnerable plaques was significantly lower than in stable plaques.</td>
</tr>
<tr>
<td>Dumont et al., 2009, USA [13]</td>
<td>Noninvasive and ex-vivo; human and phantom (18)</td>
<td>ARFI * ; quantitative</td>
<td>B-mode</td>
<td>Popliteal</td>
<td>The investigated method can be used in deep vessels such as the popliteal.</td>
</tr>
<tr>
<td>Rammarine et al., 2014, UK [20]</td>
<td>Noninvasive; human (81)</td>
<td>Shear wave; quantitative</td>
<td>Neurological symptoms</td>
<td>Carotid</td>
<td>Elastography was able to assess plaque stiffness when compared to histology. May serve as an adjunct to B-mode.</td>
</tr>
<tr>
<td>Liu et al., 2014, China [15]</td>
<td>Noninvasive; human (19)</td>
<td>Strain elastography; qualitative (visual score)</td>
<td>Histology</td>
<td>Carotid</td>
<td>Elastography was able to assess plaque stiffness when compared to histology.</td>
</tr>
<tr>
<td>Okimoto et al., 2006, Japan [16]</td>
<td>Noninvasive; human (242)</td>
<td>ARFI * ; quantitative</td>
<td>Cardiovascular risk factors</td>
<td>Carotid</td>
<td>Carotid artery elasticity was associated with increasing number of risk factors.</td>
</tr>
<tr>
<td>Wang et al., 2014, USA [17]</td>
<td>Noninvasive; human (24)</td>
<td>Strain elastography; semi-quantitative</td>
<td>Cognitive performance</td>
<td>Carotid</td>
<td>There is an association between axial and lateral strain and cognitive impairment.</td>
</tr>
<tr>
<td>Takimura et al., 2014, Japan [18]</td>
<td>Noninvasive; human (65)</td>
<td>Strain elastography; semi-quantitative</td>
<td>Endovascular evaluation</td>
<td>Femoral and popliteal</td>
<td>Elastography is able to detect the hardness of plaques.</td>
</tr>
<tr>
<td>Ribbers et al., 2006, The Netherlands [19]</td>
<td>Noninvasive and ex-vivo; human and phantom (12)</td>
<td>Strain elastography; semi-quantitative</td>
<td>Histology</td>
<td>Carotid</td>
<td>2D elastography is feasible on carotid artery plaques.</td>
</tr>
<tr>
<td>de Korte et al., 2002, The Netherlands [23]</td>
<td>Invasive; human (12)</td>
<td>Strain elastography; semi-quantitative</td>
<td>Histology</td>
<td>Coronary</td>
<td>Significantly higher strain values in non-calcified than in calcified plaques.</td>
</tr>
<tr>
<td>Keshavarz-Motamed et al., 2014, Canada [21]</td>
<td>Invasive; human (12)</td>
<td>Strain elastography; semi-quantitative</td>
<td>Histology</td>
<td>Coronary</td>
<td>Significantly lower strain values in arteries after atherectomy.</td>
</tr>
<tr>
<td>de Korte et al., 2002, The Netherlands [22]</td>
<td>Animal; pigs (6)</td>
<td>Strain elastography; semi-quantitative</td>
<td>Histology</td>
<td>Iliac and femoral</td>
<td>There are higher average strain values for fatty compared with fibrous plaques.</td>
</tr>
<tr>
<td>Zhang et al., 2010, China [5]</td>
<td>Animal; pigs (7)</td>
<td>Strain elastography; semi-quantitative</td>
<td>Histology</td>
<td>Renal arteries</td>
<td>The extent of macrophage infiltration is correlated positively with strain values.</td>
</tr>
<tr>
<td>Hu et al., 2011, China [24]</td>
<td>Animal; rabbits (40)</td>
<td>Strain elastography; semi-quantitative</td>
<td>Histology</td>
<td>Abdominal aorta</td>
<td>There are significantly higher strain values for fatty plaques compared with fibrous or fibro-fatty plaques.</td>
</tr>
<tr>
<td>Liang et al., 2009, USA [25]</td>
<td>Ex-vivo and in-vivo; pigs and phantom (3)</td>
<td>Strain elastography; qualitative</td>
<td>Histology</td>
<td>Coronary</td>
<td>Endovascular elastography is able to identify some vulnerable plaque features.</td>
</tr>
<tr>
<td>Majdouline et al., 2013, Canada [26]</td>
<td>Ex-vivo and in-vivo; pigs and phantom (8)</td>
<td>Strain elastography; quantitative and qualitative</td>
<td>Histology</td>
<td>Both carotid</td>
<td>Strain values are positively correlated with plaque condition defined by American Heart Association.</td>
</tr>
<tr>
<td>Schaar et al., 2003, The Netherlands [27]</td>
<td>Ex-vivo; human (24)</td>
<td>Strain elastography; semi-quantitative</td>
<td>Histology</td>
<td>Coronary</td>
<td>Plaques assessed as vulnerable on elastography had a thinner cap.</td>
</tr>
<tr>
<td>de Korte et al., 2000, The Netherlands [28]</td>
<td>Ex-vivo, invasive; human (13)</td>
<td>Strain elastography; semi-quantitative</td>
<td>Histology</td>
<td>Femoral and coronary</td>
<td>Different strain values were reported for fibrous, fibro-fatty and fatty plaques.</td>
</tr>
<tr>
<td>Majdouline et al., 2013, Canada [26]</td>
<td>Ex-vivo and in-vivo; phantom and pigs (8)</td>
<td>Strain elastography; quantitative and qualitative</td>
<td>Histology</td>
<td>Both carotid</td>
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<td>Liang et al., 2009, USA [25]</td>
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<td>Strain elastography; qualitative</td>
<td>Histology</td>
<td>Coronary</td>
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<tr>
<td>Ribbers et al., 2006, The Netherlands [19]</td>
<td>Ex-vivo; phantom and human (12)</td>
<td>Strain elastography; semi-quantitative</td>
<td>Histology</td>
<td>Carotid</td>
<td>2D elastography is feasible on carotid artery plaques.</td>
</tr>
<tr>
<td>Rammarine et al., 2014, UK [14]</td>
<td>Ex-vivo; phantom</td>
<td>Shear wave; quantitative</td>
<td>Validation in phantom</td>
<td>SSWE can quantify YM in carotid artery phantoms.</td>
<td></td>
</tr>
<tr>
<td>Dumont et al., 2009, USA [13]</td>
<td>Noninvasive; phantom and human (18)</td>
<td>ARFI * ; quantitative</td>
<td>B-mode</td>
<td>Popliteal</td>
<td>The investigated method can be used in deep vessels such as the popliteal. artery</td>
</tr>
</tbody>
</table>

* Early non-commercial ARFI method that uses an ultrasonic push-pulse for displacement and measures tissue displacement by speckle tracking

§ Studies that evaluate strain values in both phantoms and other subjects. They are therefore presented more than once in respective subgroups
of the duplex ultrasonography estimation are gender differences, high carotid bifurcation, obesity and in situ carotid stents. Magnetic resonance imaging is another noninvasive method, which has high diagnostic accuracy for fatty vulnerable plaques [32–34], but is very expensive and time-consuming for wide spread clinical use [35, 36]. It has been suggested that further risk stratification of atherosclerotic plaques may improve patient outcome. Ultrasound elastography may help in this risk stratification. Most noninvasive studies of ultrasound elastography have investigated strain elastography in the carotid arteries and reported higher strain values or described vulnerable plaques as soft [12, 14, 15]. One study reported lower strain values for vulnerable plaques utilizing a non-commercially available strain measuring method [11]. This finding is in contradiction to other findings both in endovascular elastography and noninvasive strain measurements [17, 23]. The reason for these contradictory findings may be a lack of consensus as to how to evaluate strain. Most studies define a plaque as vulnerable if localized high strain spots are found. One of the included studies reported mean values over entire plaques and contrary to other studies failed to find an association between high strain values and vulnerable plaques. This was the only study to use MRI as the method of reference [11].

Endovascular elastography studies in-vivo and ex-vivo have demonstrated the ability to differentiate between fibrous plaques and fatty plaque components [23, 28]. Furthermore, the ability to detect vulnerable areas in heterogeneous plaques was demonstrated, i.e., fatty regions with an increased macrophage content were located on the basis of high strain values [21]. One study provided further evidence by demonstrating lower strain values in the coronary arteries after an atherectomy was performed [21]. Although endovascular elastography studies showed promise in the assessment of plaques, they were limited by the small number of subjects and this technique still needs to be validated in a larger prospective clinical setting. One central problem with endovascular elastography is the acquisition of data in a pulsating artery located in a contracting heart. There is a risk of catheter movement and thereby a mismatch between the data acquired at the low and high pressure of a cardiac cycle. This can partly be avoided by systematically obtaining values at a specific time point in the cardiac cycle. Other intravascular modalities primarily describing morphological and histological qualities of plaques such as optical coherence tomography and ultrasound-based virtual histology have been developed [37, 38], but they also lack evaluation in larger clinical studies [39–43].

All animal studies describe an association between high strain values and vulnerable plaques. The studies on elastography in animals are limited by the low number of investigated subjects ranging from 6 pigs to 40 rabbits [22, 24] and were performed to evaluate mathematical models for elastography. Another limitation of these studies is the phenotype of the plaques, which is homogeneous contrary to the heterogeneous plaques seen in patients due to very controlled diets and environmental settings for animals. Most of the studies only reported strain values for early fatty lesions [22] and in one case no fatty lesion was observed which can be partly explained by the short timeframe of the study [5].

Ex-vivo studies with excised vessels indicate that elastography is a reliable method for the estimation of plaque morphology and thereby risk [27, 28]. Currently available elastography methods have been proven feasible in phantom studies [13, 14, 19]. However, techniques can still be optimized and more consistent reporting of the reliability of the methods used is desirable. Additional information could be gained by larger prospective controlled trials with endpoints such as mortality and morbidity for both endovascular and noninvasive modalities. Studies with a longer follow-up time could add information on the development of vulnerable plaques and their strain values or shear wave speed. Recent papers bring light to the relationship between strain in plaques and the stability of plaques for noninvasive elastography [12]. However, these strain values have not been correlated to patient outcome. Therefore, there is a need for prospective studies describing the relationship between strain values and patient outcome in a clinical setting, and noninvasive elastography would be the best way to start, as it does cause any harm to patients. Moreover, the technology is easily accessible in most hospitals with high-end equipment. Once the technology has been validated noninvasively, further endovascular studies would be appropriate. So far endovascular studies have been limited by the small number of subjects and few research groups. Future endovascular studies would require a larger cohort to provide additional information in the field. Interventional endovascular studies describing elasticity in arteries before and after an intervention would be welcome. To date, only one study has described this [21].

**Limitations**

Different issues limit the comparability of the present studies. Very few studies reported if any blinding was employed between investigators measuring strain and investigators defining plaques as vulnerable. In general, studies were heterogeneous.

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**Table 2** The elastography methods used in the included studies.

<table>
<thead>
<tr>
<th>Property measured/displayed</th>
<th>Strain elastography</th>
<th>Acoustic radiation force impulse imaging</th>
<th>Shear wave elastography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Displacement/stimulation of tissue obtained through</td>
<td>Manual compression using US transducer or physiological movement, i.e., pulsation</td>
<td>Focused radiation force impulse</td>
<td>Focused radiation force impulse</td>
</tr>
<tr>
<td>Property measured/displayed</td>
<td>Tissue strain</td>
<td>Tissue displacement</td>
<td>Shear wave speed</td>
</tr>
<tr>
<td>Quantitative or qualitative data</td>
<td>Qualitative (semi-quantification may be applied)</td>
<td>Qualitative (semi-quantification may be applied)</td>
<td>Quantitative</td>
</tr>
<tr>
<td>Real-time/still frames</td>
<td>Real-time</td>
<td>Still frames</td>
<td>Real-time</td>
</tr>
</tbody>
</table>

$\text{§} \quad \text{The acoustic radiation force impulse method used in some of the included studies and described here is different from the method usually referred to as ARFI, which is a method used for point measurements of shear wave speed by placing an ROI on the B-mode image.}$

$\text{#} \quad \text{The shear wave elastography method described here is the trademark of Supersonic Imaging (Aix-en-Provence, France), which uses a plane wave technique, which allows shear wave elastograms to cover the entire B-mode image and be refreshed at the same frame rate as the B-mode image.}$

$\text{*} \quad \text{Tissue strain is inversely proportional to the Young’s modulus (E), which is a measure of tissue elasticity, following the equation: Y = \text{stress} / \text{strain}, where the stress is the force applied to compress the tissue. Tissue strain is unitless.}$

$\text{§} \quad \text{The Young’s modulus (E) is proportional to the square of the shear-wave speed (s_c): E = 3 \times \rho \times s_c^2, where \rho is the tissue density.}$

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and the outcomes therefore differed [11,27]. There were no prospective intervention studies showing a better outcome if elastography was utilized in a clinical setting. No cut-off values for strain or shear wave elastography have been established and values may differ between different manufacturers of ultrasound equipment. Included patient groups differ between studies and the overall number of included patients is too low to evaluate the efficacy of elastography in a clinical setting.

**Conclusion**

Elastography is a feasible modality to distinguish between vulnerable and stable plaques in atherosclerosis. There is, however, a lack of larger prospective studies examining the efficacy of this technique in a clinical setting and the impact on mortality and morbidity. Current results show promise for this technique to be utilized in clinical studies evaluating the performance of new drugs for the reduction of plaque burden.

**References**


