Assessment of spatial inhomogeneities in intima media thickness along an arterial segment using its dynamic behavior

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We assessed spatial inhomogeneities in intima media thickness (IMT) along an arterial segment using its dynamic behavior. The inhomogeneities were revealed by spatial variance significantly larger than the temporal variance over several beats. If differences between positions were confirmed, the extent of the inhomogeneity was obtained by comparison of IMT and ΔIMT at each position with their spatial medians ± the least-significant difference. Because no intersubject comparisons were necessary, a single session of several measurements was sufficient to assess inhomogeneities in the arterial wall properties of a subject, making the method independent of biological variability between subjects. The method was evaluated on 47 presumed healthy subjects (age range 21–75 yr). In 22 subjects, spatial inhomogeneities in ΔIMT occurred (P < 0.05). In young subjects, ΔIMT was locally decreased, i.e., in systole inhomogeneities were less compressed than their surrounding tissue. In older subjects, ΔIMT was locally increased, i.e., the inhomogeneity was locally more compressed than its surrounding wall tissue.

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In the early development of cardiovascular disease (CVD), arteries first become more elastic, whereafter they stiffen (11, 20). Assessing these locally changed structural and mechanical properties noninvasively in a single subject is difficult mainly due to the small spatial and temporal changes involved. Even at more advanced stages, changes in arterial wall properties are hard to determine because most methods suffer from low sensitivity and specificity. For instance, even though an increased intima media thickness (IMT) of the common carotid artery (CCA) marks changing vessel wall properties and seems to be a potential candidate to predict the future development of CVD (15, 21), the correlation between increased carotid IMT and CVD is weak. This weak correlation is a result of CVD being a focal phenomenon confined to the intima with a relatively small thickness (~2.5% of IMT). Only in more diseased arteries does the intima constitute >20% of the IMT. The correlation between IMT and CVD is further attenuated due to the physiological effects of aging (16). Hence, carotid IMT has to be significantly increased (~1.2 mm) to be conclusive (1). To enhance sensitivity, IMT is analyzed along an arterial segment and several measurements are averaged (3). However, recent lesions, fatty streaks, more advanced lesions (atheroma), and fully developed complicated plaques tend to be localized at specific sites (2), resulting in only a locally deviating IMT, which is obscured by averaging over relatively long arterial segments.

The low specificity and sensitivity of methods in determining arterial wall properties usually originate from large intersubject variations and low precision of the employed method in relation to the stage of the disease. To get a more specific and sensitive indicator, either multiple properties have to be determined simultaneously (9) or one property has to be determined several times separated either in space or time. The ankle arm pressure index is an example of a simultaneously assessed property separated in space (23). The advantage of this method is that random physiological fluctuations, e.g., in pressure, are canceled. However, a unique cutoff to ascertain CVD is difficult to define, mainly due to the large spatial separation of measurement sites and biological variability between subjects (12, 22). Endothelial dysfunction of superficial arteries is an example of a temporally separated determined property at a specific position. It can be assessed by a comparison of endothelium-dependent changes in diameter due to ischemic occlusion and endothelium-independent dilatation due to administration of sublingual glyceryl trinitrate (7). However, also in this case, a clear cutoff value for CVD is not available, again mainly due to biological variability between subjects (14).

It can be anticipated that early changes in the structural properties of the arterial wall are revealed by the IMT and especially the change in IMT during the...
cardiac cycle (ΔIMT). Moreover, spatial heterogeneity in the structural properties of the arterial wall is expressed by the spatial variance in IMT and ΔIMT. Additionally, the extent of the spatial variance in IMT or ΔIMT over a short arterial segment reveals whether the change in elasticity is a local phenomenon at the site of the developing lesion (spatial inhomogeneity) or involves the total arterial tree (18). To avoid definition of a global cutoff value, sensitive to intersubject variations, the temporal variance in IMT or ΔIMT at one position is used as an estimate for the assessment precision, i.e., a cutoff value above which the spatial variance indicates possible inhomogeneities.

It was the aim of this study to determine whether local inhomogeneities can be assessed by comparison of simultaneously obtained spatial and temporal variances. Therefore, IMT and ΔIMT were assessed over a short arterial segment at 16 positions covering 15.8 mm. The spatial variance over multiple positions was compared with the temporal variance over several beats using ANOVA. If heterogeneity was confirmed, the extent of the lesion (inhomogeneity) was obtained by comparing the deviating IMT and ΔIMT with their medians ± the least significant difference (LSD). The proposed method was evaluated on 47 presumed healthy subjects.

MATERIALS AND METHODS

**IMT waveform measurement.** With the use of a 7.5-MHz linear array transducer and fast B-mode, the wall movement of an artery segment of 15.86 mm was assessed at 16 adjacent positions simultaneously (18). The spatial sample distance of 1.06 mm matched the beam width in the focal point. Briefly, the frame rate of an echo system (Pie Medical 350; Maastricht, The Netherlands) was increased to 651 Hz by increasing the pulse repetition frequency from 6,944 to 10,416 Hz, by increasing the interspacing between individual B-mode lines with a factor 4 and by utilizing only 50% of the transducer length. With the use of a specially developed data-acquisition system, the radio frequency (RF) data of each line were stored in the memory of a personal computer. The envelope of 16 RF lines was displayed on the screen of the ultrasound scanner, providing a real-time B-mode image. Wall positions were determined for each R wave top peak of the ECG. The high frame rate allowed cross-correlation of subsequently recorded RF lines, resulting in high-precision wall tracking (5, 6). The depth window size for the correlation procedure was set to the resolution of the system, i.e., 0.3 mm, equivalent to eight sample points at a sampling frequency of 21.3 MHz. The temporal window size was set to seven frames, converting to a temporal resolution of 10 ms at a frame rate of 651 Hz (18). Because the underlying RF lines in a B-mode image are registered sequentially, a time skew between registered distension waveforms exists. To compensate for this time skew, images were made orthogonal with respect to time by linear interpolation (17).

The change in diameter (d) as a function of time t at position p [d(p,t)] was obtained by placing tracking windows at the adventitia-media interface of the anterior wall and the media-adventitia interface of the posterior wall (Fig. 1A). The IMT waveform [IMT(p,t)] was obtained by positioning the tracking windows at the lumen-intima and media-adventitia interface of the posterior wall (Fig. 1B). Because of the passing pulse pressure, the diameter increases from a minimum to a maximum, whereas IMT decreases from a maximum to a minimum. The maximum diameter between two ECG triggers defines the peak-systolic diameter, whereas the minimum in diameter between the ECG trigger and peak-systolic diameter defines the end-diastolic diameter. The time points at which peak-systolic and end-diastolic diameters are obtained also determine the peak-systolic and end-diastolic IMT (dotted lines in Fig. 1). Subtraction of end-diastolic IMT from IMT(p,t) results in the change in IMT during the cardiac cycle for each position p [ΔIMT(p,t); Fig. 1C]. As a result of our definitions, maximum diameter changes have positive values, whereas ΔIMT have negative values. The above calculations were performed for each line separately.
Statistical analysis. A space- and time-dependent observation of IMT or ΔIMT, \( q_{pm} \), can be expressed as
\[
q_{pm} = \mu + e_{pm} + \alpha_p + \beta_m
\]
in which \( \mu \) is the overall mean, \( e_{pm} \) is the random error term (noise), \( \alpha_p \) is the change in \( q_{pm} \) associated with position \( p \) (spatial variation), and \( \beta_m \) is the change in \( q_{pm} \) associated with measurement \( m \) (temporal variation). It was assumed that \( e_{pm}, \alpha_p, \) and \( \beta_m \) are normally zero mean distributed and that no interaction between the different terms exists. The random error term, \( e_{pm} \), can be interpreted as the precision of the system to determine IMT or ΔIMT at any time or position. The random error term is clearly visible in Fig. 1 as the beat-to-beat stepwise transition in IMT and the system to determine IMT or ΔIMT, which is caused by the determination of the wall position for each beat (Fig. 1). The variance in IMT or ΔIMT over time depends on \( e_{pm} + \beta_m \), whereas the variance in IMT or ΔIMT over space depends on \( e_{pm} + \alpha_p \). In the absence of any spatial and temporal variations, both \( \alpha_p \) and \( \beta_m \) are zero. The temporal variation, \( \beta_m \), depends on beat-to-beat physiological variations, e.g., the change in pressure due to breathing (≈0.3 Hz) or the baroreflex (≈0.1 Hz), and is shown in Fig. 1 as superimposed on the stepwise transition. If the contribution of the temporal variation to the temporal variance is small, the temporal variance can be used as an estimate for \( e_{pm} \). If a spatial inhomogeneity exists, \( \alpha_p \) increases and the ratio of spatial and temporal variance is larger than unity. A significantly increased spatial-to-temporal variance ratio can be used as an indication for inhomogeneities. The computational procedure to obtain spatial and temporal variances and the test for significance are explained below.

The temporal variance follows from the average of the variances over all measurements at each position, that is
\[
\text{temporal variance} = \frac{1}{n_p} \sum_{m=1}^{n_m} \left( \frac{1}{n_m - 1} \sum_{m=1}^{n_m} (q_{pm} - \bar{q}_p)^2 \right)
\]
in which \( \bar{q}_p \) is the average over all measurements at position \( p \), \( n_m \) is the number of considered measurements, and \( n_p \) is the number of positions along which the parameter is determined (18). The spatial variance can be obtained by determining the average of the variances over all positions during each measurement, that is
\[
\text{spatial variance} = \frac{1}{n_m} \sum_{m=1}^{n_m} \left( \frac{1}{n_p - 1} \sum_{p=1}^{n_p} (q_{pm} - \bar{q}_m)^2 \right)
\]
in which \( \bar{q}_m \) is the average over all positions during measurement \( m \). Assuming normally zero-mean distributed \( e_{pm}, \alpha_p, \) and \( \beta_m \), and no interaction between the different terms, the spatial variance can also be obtained from the variation in \( \bar{q}_p \)
\[
\text{spatial variance} = \frac{n_m}{n_p - 1} \sum_{p=1}^{n_p} (\bar{q}_p - \bar{q})^2
\]
in which \( \bar{q} \) is the overall mean. Equation 3 determines the mean of the variances, whereas Eq. 4 determines the variance of the mean.

The ratio of Eqs. 2 and 4 results in an \( F \) distribution allowing the use of ANOVA to compare the spatial and temporal variance (19). \( F \) values were obtained according to
\[
F_{np(nm-1)} = \frac{\text{spatial variance}}{\text{temporal variance}}
\]
\( F \) values larger than a critical \( F \) value indicate that one or more positions are significantly different from the others, a clear indication of a locally deviating arterial wall property. Critical \( F \) values can be obtained from the \( F \) distribution with a significance level of 0.05 and \( n_p - 1 \) and \( n_m(n_m - 1) \) degrees of freedom for the spatial and temporal variances, respectively. \( F \) values and critical \( F \) values were obtained for each subject individually.

In the above calculations, all positions are compared with each other, i.e., it is determined whether minimum and maximum values differ significantly from each other. To exclude those results with an accidentally large difference between minimum and maximum, values must also be within the median ± the least significant difference (LSD). The LSD can be obtained from
\[
LSD = t_{np(nm-1)0.05} \times \sqrt{\frac{2 \times \text{temporal variance}}{nm}}
\]
in which \( t_{np(nm-1)0.05} \) is the Student’s \( t \)-test value with \( n_p(n_m - 1) \) degrees of freedom and a significance level of 0.05 (19). The LSD constraint increases sensitivity.

Study subjects. Validation of the proposed method was performed through an in vivo study in 47 (29 women and 18 men) presumed healthy volunteers ranging in age from 21 to 75 yr (mean age 49 ± 13 yr). All subjects gave written informed consent to participate in the study, which was approved by the joint medical ethical committee of the Academic Hospital Maastricht and the University Maastricht. The site of measurement was a straight longitudinal section of the left CCA with the most distal RF line 2–3 cm proximal to the brachial artery. DBP and SBP, diastolic and systolic blood pressure, respectively.

| Table 1. Intersubject properties of CCA of male and female subjects subdivided in groups below and above 50 yr |
|---|---|
| Male Subjects (n = 18) | Female Subjects (n = 29) |
| n | 10 | 15 |
| Age, yr | 37 ± 8 | 39 ± 9 |
| Diameter, mm | 7.1 ± 0.6 | 6.6 ± 0.5 |
| Distension, mm | 0.50 ± 0.14 | 0.44 ± 0.19 |
| IMT, mm | 0.54 ± 0.15 | 0.56 ± 0.09 |
| ΔIMT, mm | −0.04 ± 0.02 | −0.04 ± 0.02 |
| DBP, mmHg | 78 ± 16 | 78 ± 10 |
| SBP, mmHg | 124 ± 20 | 121 ± 15 |
| D >50 yr (n = 22) | 8 | 14 |
| n | 62 ± 7 | 60 ± 7 |
| Diameter, mm | 7.6 ± 0.8 | 7.0 ± 0.6 |
| Distension, mm | 0.36 ± 0.06 | 0.35 ± 0.13 |
| IMT, mm | 0.71 ± 0.18 | 0.73 ± 0.13 |
| ΔIMT, mm | −0.06 ± 0.03 | −0.05 ± 0.02 |
| DBP, mmHg | 88 ± 9 | 79 ± 9 |
| SBP, mmHg | 144 ± 13 | 128 ± 19 |

Values are means ± intersubject SD; n, no. of subjects. Diameter, distension, intima media thickness (IMT), and the change in IMT during the cardiac cycle (ΔIMT) were obtained in the common carotid artery (CCA) using ultrasound. Blood pressure was determined in the brachial artery. DBP and SBP, diastolic and systolic blood pressure, respectively.
to the bifurcation and the scan plane perpendicular to the plane of the bifurcation as established with the echo system. In each subject, six independent measurements were made. The recording time of each measurement was 6 s, covering at least five cardiac cycles (intersubject average 6.4). An ECG was recorded simultaneously with the B-mode measurement. The pulse pressure was determined in the left brachial artery using an oscillometric blood pressure meter (Omron 705CP). IMT and ΔIMT obtained for all beats in one measurement were averaged, resulting in six measurements consisting of end-diastolic IMT and ΔIMT determined at 16 positions.

RESULTS

Figure 1 shows the diameter (A), IMT (B), and ΔIMT (C) waveforms as a function of time for three cardiac cycles for a 36-yr-old male subject. The thin solid lines are the waveforms for all 16 positions, whereas the thick solid line is the average over all positions. End-diastolic and peak systolic diameter are indicated by the vertical dotted lines (first beat only). These lines also indicate the end-diastolic and peak-systolic IMT, respectively. Intersubject end-diastolic diameter, distension, IMT, and ΔIMT are summarized in Table 1, subdivided into male and female subjects and ages below and above 50 yr. The temporal variation was visible as a transition in diameter and IMT at the beginning of the second and third beat (Fig. 1, A and B). The distribution of the individual waveforms in Fig. 1 shows the spatial variance in diameter, IMT, and ΔIMT. Median intrasubject spatial and temporal SDs in end-diastolic diameter, distension, IMT, and ΔIMT are summarized in Table 2. The median intrasubject temporal SD indicates the precision of the system to assess IMT (80 μm) and ΔIMT (30 μm).

The IMT increase per year for younger subjects (21–50 yr) was lower than that for the older subjects (Fig. 2A). The spatial (open circles) and temporal (solid circles) variances in IMT are displayed as a function of age in Fig. 2B. Figure 2C presents the F values (open squares) and critical F values (solid line). The number of positions (N) different from the median IMT (I) determined at 16 positions is depicted in Fig. 2D. A negative value for N indicates a locally smaller IMT, whereas a positive N indicates a locally larger IMT. Although statistics indicated that there were several subjects with significantly different IMT (i.e., F value > critical F value), there were only two subjects with a difference larger than one LSD from the median, i.e., N <> 0. For these two subjects, the IMTs as a function of position are displayed in Fig.

<table>
<thead>
<tr>
<th>Male Subjects</th>
<th>Female Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter, mm</td>
<td>7.1 ± 0.10(0.10)</td>
</tr>
<tr>
<td>Distension, mm</td>
<td>0.50 ± 0.04(0.03)</td>
</tr>
<tr>
<td>IMT, mm</td>
<td>0.54 ± 0.09(0.08)</td>
</tr>
<tr>
<td>ΔIMT, mm</td>
<td>-0.04 ± 0.05(0.03)</td>
</tr>
<tr>
<td>Diameter, mm</td>
<td>7.6 ± 0.13(0.11)</td>
</tr>
<tr>
<td>Distension, mm</td>
<td>0.36 ± 0.05(0.04)</td>
</tr>
<tr>
<td>IMT, mm</td>
<td>0.71 ± 0.14(0.10)</td>
</tr>
<tr>
<td>ΔIMT, mm</td>
<td>-0.06 ± 0.07(0.03)</td>
</tr>
</tbody>
</table>

Values are medians ± intrasubject spatial SD and intrasubject temporal SD (in parentheses).
3, A and B, respectively. The arrows indicate the positions that are significantly different from the median IMT. Visual inspection of the IMT for all other subjects revealed no inhomogeneities.

Figure 4A displays ΔIMT as a function of age. There was a tendency for ΔIMT to become larger beyond 50 yr. Figure 4B shows a larger spatial variance (open circles) compared with the temporal variance (solid circles). The precision in determining ΔIMT was estimated at 30 μm (equal to the median temporal SD; Table 2). The better precision in determining ΔIMT compared with IMT (∼80 μm) was a result of the cross-correlation procedure between RF lines, which determines the displacements of walls by estimation of the phase shift between two successively recorded B-mode frames (5, 6, 18). The larger spatial variance was also expressed by the larger $F$ values (open squares, Fig. 4C) compared with the critical $F$ values (solid line, Fig. 4C) and the number of positions, $N$, that are significantly different from the median ΔIMT (Fig. 4D).

Figure 5A shows a subject having five positions (indicated by the arrows) a significantly larger ΔIMT, whereas Fig. 5B shows a subject having at two positions a significantly lower ΔIMT.

DISCUSSION

To reveal spatial inhomogeneities in structural and mechanical properties of an arterial wall, the spatial intrasubject variances in end-diastolic IMT and ΔIMT along the arterial segment were compared with the temporal variance using ANOVA. Significantly increased spatial variance indicates locally deviating...
a 62-yr-old male subject with a significantly increased IMT can be obtained from the number of positions these spatial inhomogeneities in end-diastolic IMT and structural and mechanical properties. The extent of with a significantly decreased IMT during the cardiac cycle, i.e., the surrounding tissue is more compressed than the site of the lesion itself. The example shown in Fig. 5A illustrates

Despite possibly large temporal variations, the sensitivity of the method was high, as illustrated by the relative large number of subjects having a spatial variance higher than the temporal variance, i.e., large F values compared with the critical F value (Fig. 2C). In comparing the spatial with the temporal variance using ANOVA, all positions were compared with each other, i.e., if the minimum and maximum were significantly different from each other, the F value became larger than the critical F value. Restricting our results only to those cases that differed significantly from the median, thus excluding accidental large differences between minimum and maximum, revealed only two subjects with an inhomogeneity in IMT (Fig. 2D). For the subject with a significantly smaller IMT at one position, IMT decreased linearly as a function of position (Fig. 3A). Important to note is that the deviating IMT (383 μm) was only 97 μm smaller than the median (480 μm), i.e., slightly exceeding the system precision (~80 μm). For the other subject, the larger IMT (~836 μm) compared with the median (676 μm) at two positions (Fig. 3B) was also visible in the original B-mode image (white arrows in Fig. 6).

Although ΔIMT is not the same as elasticity, it can be envisaged that it will change when artery wall properties are altered (8, 10). Intrasubject comparison of spatial with temporal variance in ΔIMT showed inhomogeneities in a surprisingly large number of subjects (Fig. 4D). At younger ages, these inhomogeneities were predominantly positive (N > 0), whereas at older ages these inhomogeneities were negative (N < 0). A positive inhomogeneity in ΔIMT indicates a locally less decreased IMT during the cardiac cycle, i.e., the surrounding tissue is more compressed than the site of the lesion. The example shown in Fig. 5A illustrates

Fig. 5. ΔIMT as a function of position for a 28-yr-old female subject with a significantly decreased ΔIMT at 5 positions (arrows in A) and a 62-yr-old male subject with a significantly increased ΔIMT at two positions (arrows in B). The thin solid lines are single measurements. The thick solid line is the average of all measurements. The dashed line indicates the median ΔIMT.

Fig. 6. B-mode image of a 62-yr-old male subject with significantly increased IMT at 2 positions (see also Fig. 3B). The arrows indicate the site of the lesion. The B-mode image consists of 16 lines and was displayed in real time on the ultrasound scanner.
that IMT locally even increases during the cardiac cycle ($\Delta IMT > 0$), suggesting possible displacement of wall mass from adjacent parts of the arterial wall to the focal site (8, 10). Other subjects with positive inhomogeneities showed similar features. Whether this type of inhomogeneity is correlated to a decrease in stiffness during early development of atherosclerosis is not clear. The negative inhomogeneities at older age showed the opposite effect, i.e., the inhomogeneity was more compressed than the surrounding wall tissue (local $\Delta IMT >$ median $\Delta IMT$). Possibly the wall mass is displaced from the focal site to the surrounding tissue or the elastic behavior of the artery is locally indeed increased, e.g., due to an accumulation of proteoglycans in the arterial wall (4).

It should be noted that the previous results do not necessarily mean that the complete circumferential wall area is compressed or stretched, because the wall material can be nonuniformly displaced around the arterial wall. Intravascular ultrasound confirmed that at sites with focal lesions the radial strain (equal to $\Delta IMT/IMT$) was decreased, whereas at other sites (in the radial direction) strain was increased, thus preserving total circumferential wall volume (10). Hence, it may be concluded that for a noncircumferential focal lesion, the plane of observation has a large influence on whether a significant compression or stretch is obtained. To determine whether a lesion is circumferential and to exclude erroneous results in determining compression of arterial walls, ultrasound observations should be made at two orthogonal directions. It can be envisaged that a noncircumferential inhomogeneity is compressed in one field of view, whereas it will be stretched in the perpendicular field of view.

The surprising results for heterogeneity in IMT and $\Delta IMT$ were obtained with high accuracy. This high accuracy was a result of sequentially applying two different statistical tests. The $F$ test compares the spatial with the temporal variance, implicitly testing the precision of the system. That is, both spatial and temporal variations depend not only on the spatial ($\epsilon_p$) and temporal ($\beta_m$) variations but also on the precision ($\epsilon_{pm}$, Eqs. 2 and 3). Decreased precision results in lower $F$ values (Eq. 5), thus lowering sensitivity to reveal inhomogeneities. Significantly increased $F$ values only indicate that there are differences among the different positions. In fact, we tested whether the extrema were significantly different from each other with a confidence level of 95%. LSD compares each individual position with the median, thus enhancing sensitivity to a confidence level of maximal 99.75%. Also, in this case, lower precision, i.e., increased temporal variance, lowers sensitivity due to increased LSD values (Eq. 6). Hence, a significantly different IMT or $\Delta IMT$ indicates inhomogeneities with a high confidence level.

Because the method as described above is independent of the echo line density in the current spatial observation window, there seems to be no objection in performing the same statistical analysis on B-mode images recorded on video, which usually have a higher line density than the fast B-mode system presented here. However, because complex cross-correlation of RF signals is not possible on video data, the observed change in IMT during the cardiac cycle will be less precise. The relative large temporal variance will decrease sensitivity for determining heterogeneities in $\Delta IMT$ considerably. Hence, only more advanced techniques to determine IMT and $\Delta IMT$ allow detection of inhomogeneities in (video) B-mode images. An advantage of video images is the larger spatial observation window, thus increasing the possibility to detect a lesion. However, the sensitivity to small lesions will decrease, due to the relatively smaller contribution of the small lesion to the spatial variance.

In conclusion, intrasubject comparison of spatial and temporal variations in IMT and $\Delta IMT$ exposed locally deviating arterial wall properties. Because no intersubject comparisons were necessary, a small set of measurements was sufficient to assess inhomogeneities in the arterial wall properties of a subject. Because of the high precision to determine end-diastolic IMT, small spatial variations in IMT could be detected with a high sensitivity. Sensitivity in determining spatial variations in $\Delta IMT$ was even higher because of the complex correlation method used to detect wall motion. For young subjects, $\Delta IMT$ was locally decreased, i.e., the surrounding is more compressed than the site of the inhomogeneity. For older subjects, $\Delta IMT$ was locally increased, i.e., the inhomogeneity was locally more compressed than its surrounding tissue.

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REFERENCES


