Peer Review File

Article Information: https://dx.doi.org/10.21037/cdt-21-160

Reviewer A:

Comment #1: please report the inter- and intra- observer variability of the NIRS measurements

Reply 1: The NIRS probe at the distal tip of the catheter acquires 40 spectroscopic measurements per second during automated pullback through the catheter at a speed of 0.5 mm/s and 16 rotations per second. Every measurement is binary coded as positive or negative. The algorithm decoding wavelength spectra into the chemogram is a propriety of the manufacturer and is not publicly available. The LCBI is a summary measure of the lipid signal along the interrogated segment of the vessel on a scale of 0 to 1000. The LCBI is calculated as a fraction of yellow pixels in the chemogram multiplied by a factor of 1000. We performed single NIRS-IVUS pullback through the stented segment of carotid artery, therefore the intraindividual variability of LCBI measurement cannot be determined. However, it has been reported in previous studies (Gardner CM et al. JACC Cardiovasc Imaging 2008; 1: 638-48., Waxman S et al. JACC Cardiovasc Imaging 2009; 2: 858-68.). The LCBI is automatically calculated from the region of interest (ROI) on the chemogram. The ROI was easily determined from the distinct appearance of the stent on the coregistered IVUS image. Because of the automated calculation of LCBI, inter- and intra- observer variability of LCBI measurement was not determined.

Changes in the text: Additional explanation for the calculation of LCBI from the chemogram was added in the methodology (page 7).

Chemograms were compared to assess the change in the lipid signal from the segment of ICA and CCA with implanted stent (i.e. region of interest – ROI) from baseline to the follow-up. The following parameters were used to quantify the lipid signal: LCBI of the entire stented segment and maximal LCBI in any 4-mm segment of the chemogram (LCBImax). LCBI and LCBImax were automatically calculated from the ROI defined by the distinct appearance of the stent on the coregistered IVUS image.

Reviewer B:

The use of NIRS-IVUS imaging for CAS is novel and an important research direction.

The primary finding is a decrease in LCBI from post stenting to a median of 31 mo FU. Most of the change in NIRS Lipid comes from a decrease in higher values of Max 4 mm LCBI and LCBI total. For the Max 4 mm data there were 6 patients with >100 a LCBI versus only 1 during FU. The response of extreme LCBIs may be quite helpful, and is worth noting. As noted the depth of the lipid signal is not known with NIRS. Could the decrease have come from intimal hyperplasia blocking the signal. This could reflect sealing.

Reply 1: We thank the reviewer for pointing out that patients with the highest LCBI had largest reduction during the follow-up. We mentioned this observation in the results. Regarding the explanation for the observed reduction of lipid signal. Indeed, stent expansion and neointimal hyperplasia leads to increased distance of the NIRS-IVUS catheter from the plaque that is sealed under the stent. We acknowledge the

limitation of the study is that plaque sealing with NIH cannot be distinguished from actual change of the plaque composition. The reduction of lipid signal without significant change in the plaque burden in coronary arteries has been described after intensive statin therapy (Dohi T et al., EHJ-CVI 2015; 16: 81–87). However, NIH has been observed on IVUS together with decreased lipid signal on NIRS and we termed this plaque sealing.

Changes in the text: Results – lipid signal, additional description of LCBI changes.
The follow-up LCBImax < 100 was present in all but one case.
There was no correlation between follow-up LCBI and luminal volume (p = 0.72) or stent volume (p = 0.34).

Discussion – expanding the paragraph explaining the concept of plaque sealing (page 10).
In our observation NIH covered the stent which was accompanied by decreased lipid signal, although the causality could not be determined due to the reasons mentioned former. The mechanism of plaque sealing might be responsible for low incidence of ipsilateral stroke after 30-days post successful CAS comparable to complete removal of the plaque with endarterectomy (2, 3).

Reviewer C:
I have read the manuscript "Long-Term Changes after Carotid Stenting Assessed by Intravascular Ultrasound and Near-Infrared Spectroscopy". In this paper, the authors reported that lipid signal derived from NIRS decreased during the follow-up period suggesting stabilization of the plaque. This clinical study has a good point of view and is well-documented, however, there are some major problems.

#1. Since NIRS-IVUS has been developed for an assessment of coronary arteries. Although recent study proved that NIRS can detect lipid rich atheroma in comparison of histological evaluation using endarterectomy
specimens of human internal carotid artery (10), it is unclear whether NIRS can detect lipid signal after further expansion by self-expanding stent in the same way as before stent expansion. In other words, the decrease in lipid signal derived from NIRS would not mean plaque stabilization, bur rather that the lipid signal may not be accurately detected due to the distance from NIRS probe. To exclude this possibility, the authors should be required to show the correlation between lipid signal and vessel diameter in ex vivo study.

Reply 1: We agree with the reviewer that NIRS signal can be obstructed by stent. In a previous study we described reduction of lipid signal immediately after CAS (Štěchovský et al., EuroIntervention 2019; 15: 289-296.). The intravascular NIRS technology has been validated on native coronary arteries and carotid atherosclerotic plaques (Kotsugi et al., Neurol Med Chir. 2020; 60: 499-506.). We recognize this as a study limitation. However, we did not observe interaction between luminal or stent volume and LCBI in a post hoc analysis. Baseline and follow-up LCBI were both detected in the same artery segment within the implanted stent, therefore there was no change in the obstruction of the signal.
The suggested ex vivo study was not performed because autopsy samples of human carotid artery could not be obtained.

Changes in the text: We performed additional analysis (correlation of luminal and sent volume with LCBI) and expanded the results – lipid signal (page 8).
There was no correlation between follow-up LCBI and luminal volume (p = 0.72) or stent volume (p = 0.34).

We also recognized the obstruction of the NIRS-IVUS signal in the limitations, although it is discussed in a former section of the manuscript.
Both ultrasound and near-infrared light can be obstructed by stent, although with IVUS we assessed processes within the stent and with NIRS signal obstruction in present both at baseline and follow-up.

#2. Despite of prospective observational study, the timing of the assessment of lipid signal by NIRS after CAS has not been fixed in this study. Since the timing of stent expansion and restenosis is vital, it would be major problem in this study.

Reply 2: The study was conducted after the routine periprocedural NIRS-IVUS has been implemented. All patients from the CAS registry at least 6 months after the procedure were invited to participate on the follow-up study. We did not observe interaction between time from CAS and percentage in-stent restenosis or stent expansion (Figure 4) with the limitation that 48/58 stents had follow-up longer than 12-months. It has been suggested that most neointimal hyperplasia occurs in the first 12 months after CAS (Willfort-Ehringer et al., J Vasc Surg 2004; 39: 728 -34., Kobayashi et al. J Am Coll Cardiol 2001; 37: 1329-34.).

Changes in the text: Results (page 8).
Time from CAS did not correlate with percentage ISR volume (Pearson's correlation coefficient r= -0.02; p = 0.88) or stent expansion (Pearson's correlation coefficient r = 0.11; p = 0.45) (Figure 4A-B).

Limitations (page 11).
Only one NIRS-IVUS follow-up imaging was performed at different time intervals from CAS (median 31 months). Longer follow-up was not correlated with higher percentage ISR volume or larger stent expansion. Therefore, the temporal trend of this processes could not be clarified. Previous study suggested that most neointimal hyperplasia occurs in the first 12 months after CAS (17, 18).

#3. Since delayed stent expansion and intima formation after CAS has been remarkably influenced by the type of stent used, it would be recommended to analyze the NIRS-IVUS data by stent type.

Reply 3: In our study, there was no interaction between stent type (Xact and Wallstent closed-cell vs. Sinus open-cell) and percentage in-stent restenosis or follow-up LCBI (Table 4). Double-layer meshed stents were not used. The sample size is limited therefore post hoc subgroup analysis of stent type or other subgroups are likely underpowered.

Changes in the text: Results – stent and luminal measurements.
No significant difference in the percentage ISR volume was observed between subgroups including stent design (open versus closed cell) (Table 4).
Results – lipid signal.

Diabetics had higher follow-up LCBI compared to patients without diabetes (p = 0.02) (Table 4). No significant difference in the follow-up LCBI was detected between other subgroups (Table 4).