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Research Article

Genetic Amniocentesis Using Atraumatic 29-Gauge Needle in Patients Having a Chorioamniotic Separation

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Abstract

Background: Chorioamniotic separation (CAS) at the time of amniocentesis is a risk factor for an elevated postprocedural complication rate after standard amniocentesis and should be excluded before amniocentesis.

Objectives: The aim of this study was to quantify procedure-related risks after amniocentesis (AC) with a 29G-needle in cases of chorioamniotic separation (CAS) and evaluation of perinatal outcome in CAS after 15 weeks' gestation (GW).

Materials and Methods: Retrospective analysis of genetic amniocentesis with an atraumatic 29G-needle after 15 completed GW in pregnancies, in which the fetal membranes were not fused yet. Included into the study were women with at least 15 completed weeks of gestations referred for second trimester amniocentesis to identify fetal chromosomal aberrations aged 16 to 44 years.

Results: 437 Amniocenteses were made in total with the 29G-needle. The median maternal age was 30 (16-44) years. 145 cases showed CAS where the distance between chorion and amnion was 0.10 - 10.02 mm at amniocentesis. 38 pregnancies were terminated, 37 of which had a genetic disorder. The risk of aneuploidy increases by the factor 2 (95% CI 1.4-2.8) in relation to CAS enlargement per 1 mm distance. No procedure-related complications during two weeks after the AC were found.

Conclusions: CAS seems to be massively underreported. Early diagnosis in case of CAS should be achieved, because CAS could be an indication of genetic abnormalities – "soft marker". With the atraumatic 29G-needle, the risk for complications after amniocentesis in CAS seems to be very low.

Keywords: Amniocentesis; Chorioamniotic Separation; So0ft Marker; 29 Gauge Needle

Introduction

In 1966, Steele and Breg [1] introduced chromosome analysis after amniocentesis. Cultured amniocytes made it possible to exclude numerical and structural chromosomal anomalies cyto-genetically, especially Trisomy 21, in pregnant women with advanced maternal age or a personal or family history of chromosomal anomalies. This procedure became more and more a standard procedure over the last four decades and today, "midtrimester amniocentesis is the most common invasive prenatal-diagnostic technique offered to pregnant women at increased risk of chromosomal abnormalities" [2,3]. Various studies examined the procedure-related complication

rate and fetal loss rates between 1% and 0.06% were found [4-6]. Due to its low complication-rate, standard amniocentesis is a relatively safe technique, fetal loss rate depending on various factors [7], especially vaginal bleeding in early pregnancy [8,9], operator experience [10] and used technique [11,12]. Chorioamniotic separation (CAS) at the time of amniocentesis is a risk factor for an elevated postprocedural complication rate after standard amniocentesis and should be excluded before amniocentesis [6]. The CAS must be differentiated between spontaneous CAS (sCAS) and iatrogenic CAS (iCAS). Physiologically, the membranes are "ultimately obliterated between 12 and 16 completed weeks of gestation (GW)" [13,14]. Certainly, a special problem is given in these pregnancies, in which the chorion and the amnion are still not fused at the end of 15 completed weeks of gestation. This separation of the fetal membranes can cause a tenting effect at standard amniocentesis causing postprocedural complications [14,15].

If there is only a small distance between the separated membranes, amniocentesis can be post-pond for one week and in most cases, the membranes will be fused then. If there is a larger distance, it could be an indication of fetal chromosomal anomalies [16,17], which may per se be associated with a higher fetal loss rate after amniocentesis [18]. Especially in those cases, an early result of genetic testing is mandatory. Termination of pregnancy is psychologically more distressing with advancing gestational age and the uncertainty is also a heavy psychological burden for the parents to be. Thus, the conflict is if amniocentesis should be performed despite the higher risk of complications or should be postponed until the membranes will be fused, receiving the result of genetic testing even later. The purpose of this retrospective, observational cohort study was to examine the procedure-related complication-rates with the 29-gauge atraumatic needle in cases with CAS. Possible complications like membrane tenting, amniotic fluid leakage, amniotic fluid loss, vaginal bleeding, (iatrogenic) preterm premature rupture of membranes (iPPROM) and total fetal loss as well as pregnancy-, maternal- and fetal outcome over the last 6 years at 145 amniocenteses with sCAS were evaluated and the perspective of atraumatic amniocentesis (29gauge (0.34 mm) pencil pointed needle) are discussed.

Materials and Methods

This is a retrospective, observational study of a convenience sample of 437 pregnant women undergoing amniocentesis with the atraumatic 29-gauge pencil pointed needle between July 2008 and May and from June 2011 till February 2017. Pregnancy outcome data were obtained from feedback sheets returned by the patients, hospital records and telephone interviews after delivery.

Participants

Included into the study were women with at least 15 completed weeks of gestations referred for second trimester amniocentesis to identify fetal chromosomal aberrations aged 16 to 44 years. The main inclusion criterion into this study was an already existent medical indication for karyotyping by amniocentesis, the use of the 29-gauge pencil and the realization of the procedure by one obstetrician. Exclusion criteria were multiple pregnancies, amniocentesis for other indication (fetal infection) and amniocentesis performed with other supplements. The protocol for the intervention and data collection was approved by the ethic committee of Martin-Luther University Halle-Wittenberg. All the procedures were conducted according to the Helsinki Declaration. Written informed consent was obtained from the patients prior to intervention. The atraumatic 29G-needle (0.34 mm x 103 mm) (HVM Medical GmbH, Rotenburg an der Fulda, Germany) was used for amniocentesis. Its "pencil-point" sharpened tip displaces the two layers of the fetal membranes a traumatically (Figures 1 & 2) [12].

Measures

At first, every participant was examined sonographically with the ultrasound machines Phillips iU22 (Philips Medical, Hamburg, Germany) or Voluson E8 and E10 Expert (GE, Milwaukee, WI, USA) to confirm the gestational age and the fetus was screened for morphological anomalies. All data were compared against published, standardized references for various parameters used in ultrasound software system (Viewpoint, GE, USA). The sCAS was defined as any visible distance between the amnion and chorion at the designated area for the puncture. Gentle pressure and quick withdrawal of the ultrasound probe was applied to identify sCAS; the small echo-negative distance between both membranes layers becomes better visible or the amniotic membrane presented fluctuations after this maneuver (Figure 1).

The women were informed about possible risks of the procedure and gave an informed written consent. The patient was asked to have an empty bladder. No local anesthesia was used. After skin disinfection the sterile ultrasound gel for biopsy (SONOGEL Vertriebs GmbH, Bad Camberg, Germany) was used. The area of the biggest amount of amniotic fluid was searched under real-time ultrasound guidance. At first, a 23G-guide-needle (stylet) was inserted with an angle of 45° at least two-thirds into the myometrium without tapping the chorioamniotic membrane [11]. The tip of the needle had to be seen by ultrasound. After that, the amniotic cavity was punctured by the 29G-needle through the stylet and the chorioamniotic membranes, being rapidly introduced across the guide-needle (Figure 1). No needle insertion was performed through the placenta. The puncture was always successful at the first attempt (Figure 2). The suctioned 15 mL of amniotic fluid showed in all cases a sufficient number of cells for karyotyping. No antibiotic prophylaxis was given to the participants. Post-procedural as well as 24 to 48 hours after amniocentesis, the fetus was examined again sonographically for positive heartbeat and to detect any possible complications like intraamniotic bleeding, retroplacental hematoma, amniotic fluid leakage or (iatrogenic) Preterm Premature Rupture of Membranes (iPPROM) and fetal

death, meaning fetal loss. All participants were instructed to avoid physical stress for the next 48 hours and to return to clinic in case of any possible complication. The fetal chromosomes were examined cytogenetically.

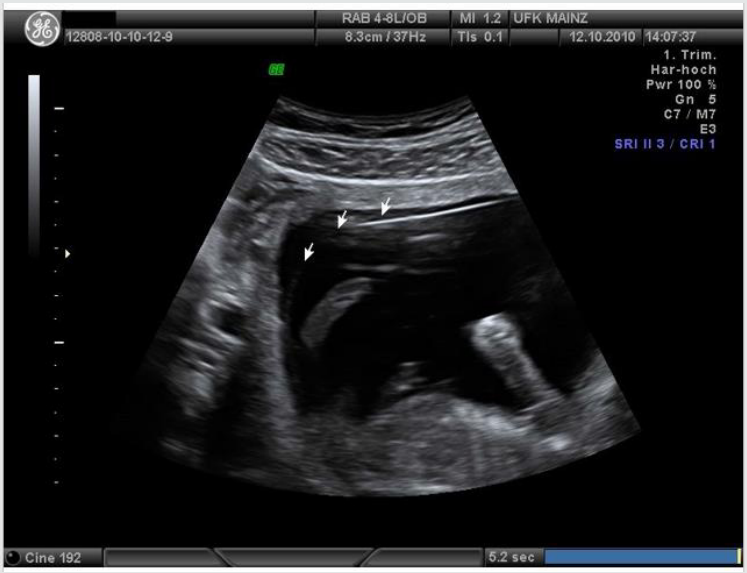

Figure 1: Chorioamniotic separation (CAS) in ultrasonography. Arrows show the CAS.

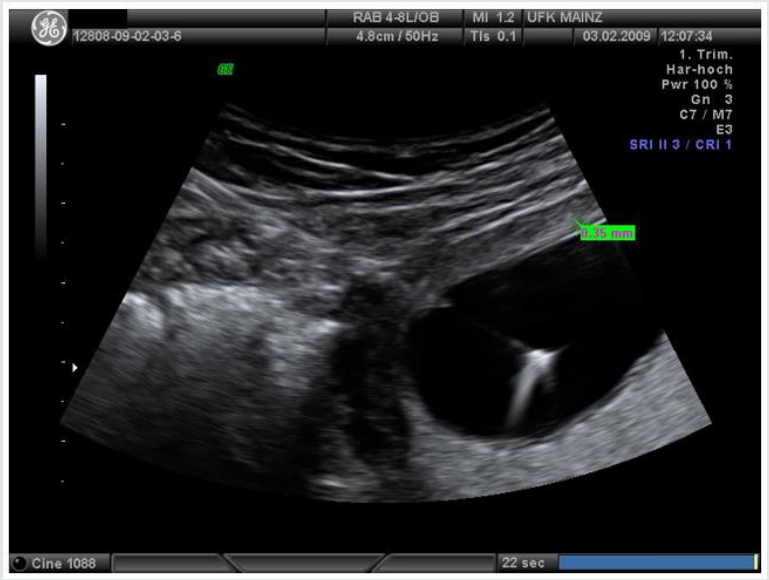

Figure 2: Chorioamniotic separation and amniocentesis with the 29G-needle in ultrasonography. The point of atraumatic 29G-amniocentesis needle is very good presented in ultrasound.

Statistical analysis

Every participant got a questionnaire at the day of examination about pregnancy and fetal outcome with the request to send it back to clinic after delivery in the case of not delivering in our clinic. Whenever possible data were obtained from hospital records. To measure the impact of the sCAS on karyotype or pregnancy outcome the odds ratio for a pathological karyotype or pregnancy outcome depending on sCAS was calculated. The sCAS was considered as a binary variable, the distance as a continuous variable and the age as categorical variable. Besides the description of the different frequencies of the variables, the dependence of karyo-type and pregnancy outcome upon CAS was recorded to identify the risk

of a pathological karyotype or an irregular pregnancy outcome in case of CAS. In this retrospective case-control-study logistic regression was used for analysis of the effect of the risk factor (CAS) on the outcome of the pregnancy (birth/termination) or the fetal karyotype (pathologic/ non pathologic). As the patient's age was included as an explanatory varia-ble it is a multivariable logistic regression.

Results

The sCAS was diagnosed in 145/437 women referred for amniocentesis after 15 GW. The outcome of 437 pregnancies was available in 399 livebirths (92 %), 38 pregnancies were terminated. The indications for amniocentesis were advanced maternal age, elevated risk for genetic anomalies at first trimester scan, personal or family history of genetic anomalies or abnormal ultrasound findings. Most patients had more than one indication. The participants were between 16 and 44 years old. The median maternal age was 30 years. The gestational age at amniocentesis was between 15+0 and 27+2 GW (Figure 3). 14 out of 37 patients of the age group (16-27) were diagnosed with sCAS (38%). An abnormal karyotype was found in 37/437 cases (8.47%) (Figure 4). 145 Amniocenteses showed a sCAS. The distance between chorion and amnion was between 0,1 mm to 10.0 mm at amniocentesis. Out of 37 patients with a pathological karyotype 68% had a sCAS in our population. The distribution of the measured distance of the CAS with its respective share of pathological or non-pathological karyotype is present in Figure 5. The risk of aneuploidy increases by the factor 2 (95% CI 1.4-2.8) in relation to sCAS enlargement per 1mm distance. No procedure-related complications during two weeks after the AC were found. The distribution of the pregnancy outcome is present in Figure 6.

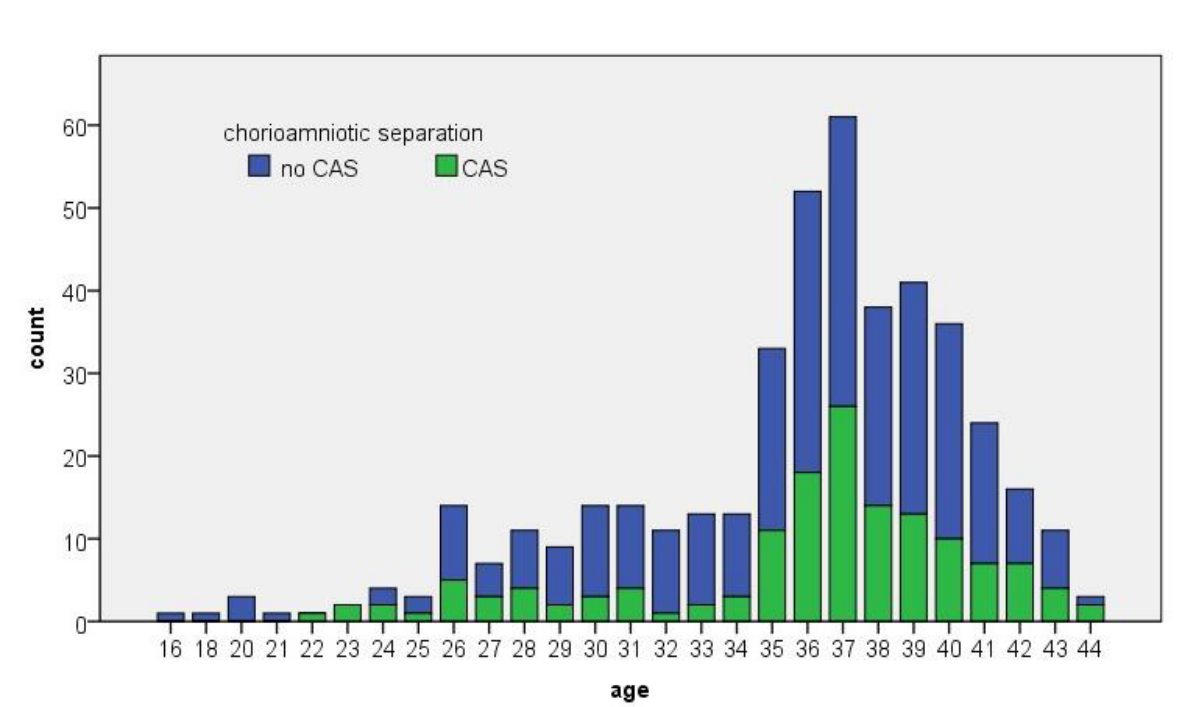

Figure 3: The age distribution of the study population with the proportion of CAS.

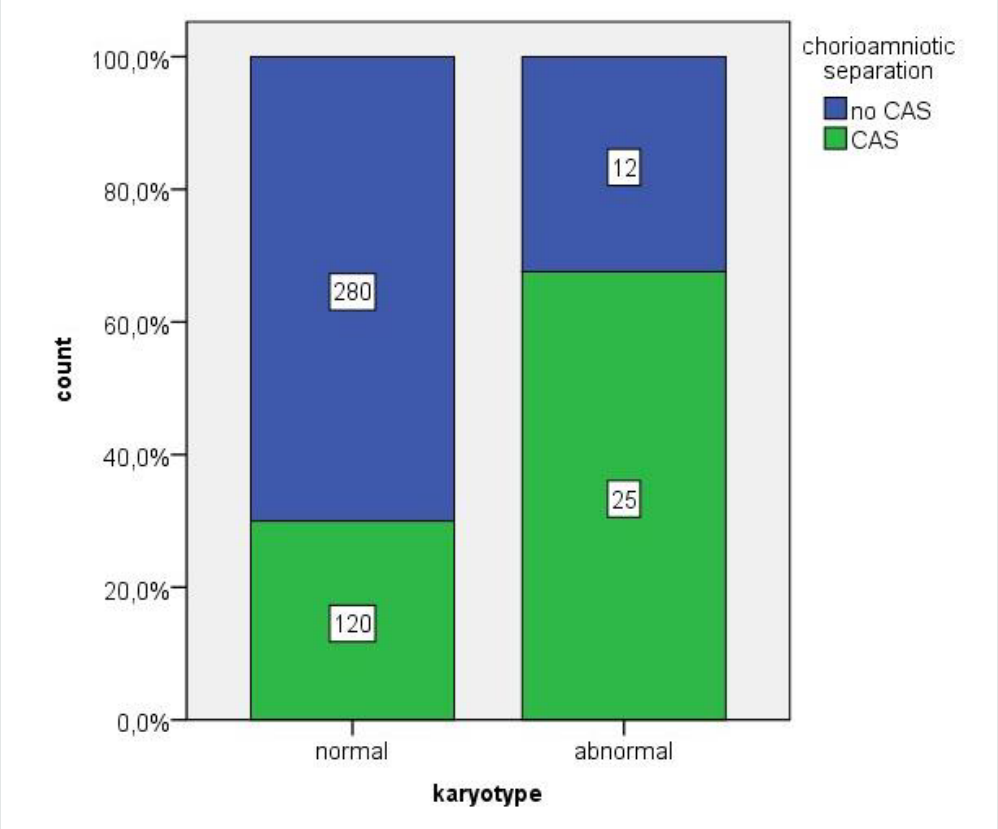

Figure 4: Frequency of CAS in correlation to the karyotype.

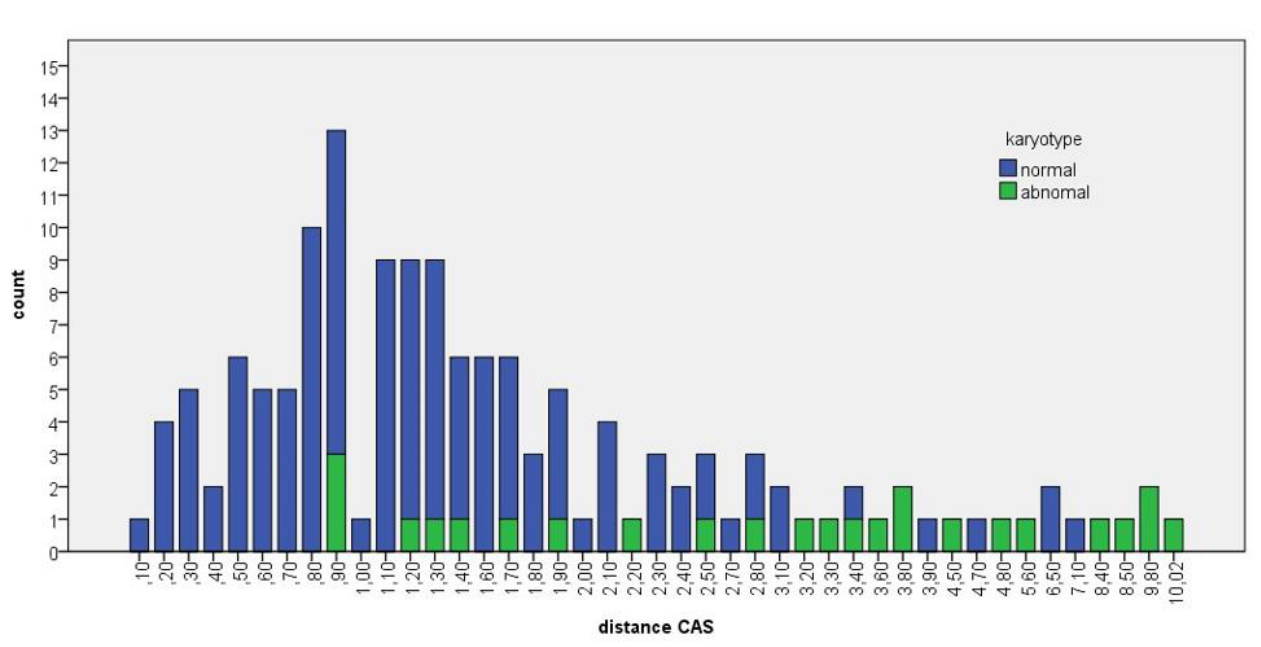

Figure 5: The distribution of pregnancy outcome in relation to CAS. The abnormal karyotype was frequently associated with the large CAS.

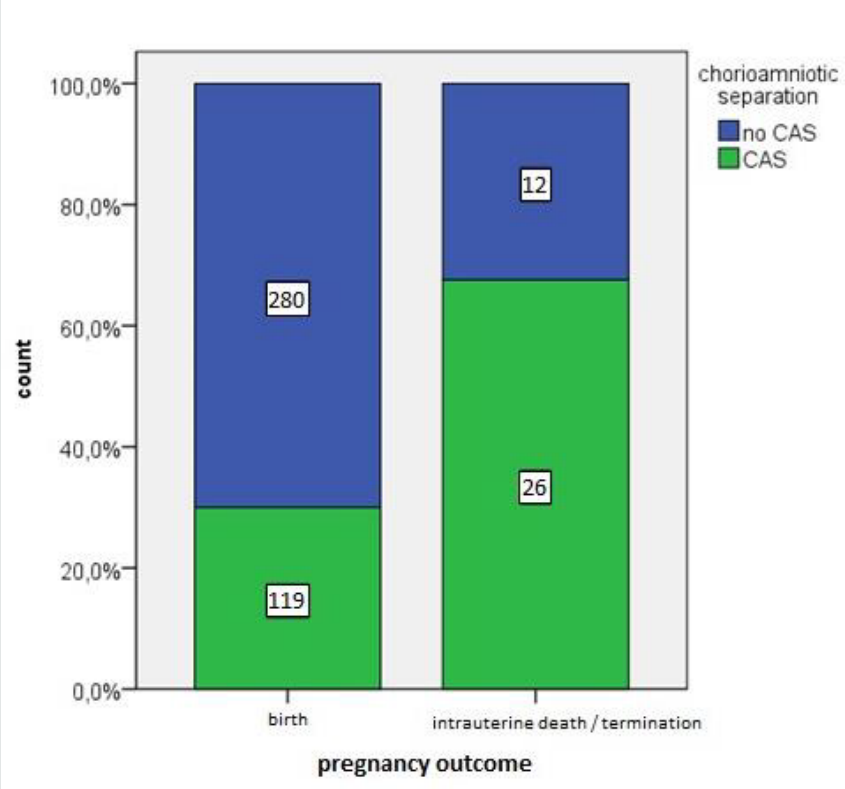

Figure 6: The distribution of the measured distance of the CAS with its respective share of pathological or non-pathological karyotype.

Discussion

To our experience sCAS does not increase the risk of miscarriage after amniocentesis using the atraumatic 29G pencil pointed needle. Due to the atraumatic 29-gauge needle there is a lower risk of damaging the amniotic sac. Due to the smaller diameter and the improved presentability of the needle tip in the ultrasound the amniocentesis is way more secure than before. The risk of fetal aneuploidy is per mm sCAS two times higher. The sufficient explanations are still not found. Possibly a pathological set of chromosomes limits the growths of fetal membranes. Human amniocytes are different and could be classified as adherent and non-adherent cells. A number of authors have referred non-adherent amniocytes as the cells with low attachment ability under routine culture conditions [19,20]. Hosseini et al. were able to identify recently four groups of amniocytes, including epithelioid, short- and long-spindle fibroblastoid cells, in addition to heterogeneous cultures containing different cell types of observations based on morphology, frequency and growth properties of amniocytes [19]. In our opinion, the heterogeneous amniociytes could have also a different reaction and functions related to aneuploidy or other genetic abnormalities. These functional and morphologic deviations of amniocytes could partly explain the sCAS. Haidi et al. also found a significantly higher frequencies of telomere aggregation in trisomy 21 amniocytes than in amniocytes from normal pregnancies reflecting the high genetic instability [21]. The other possible pathway of the sCAS could be explained by early bleedings or splits in the amniotic membranes leading to an accumulation of fluids between the two layers. After absorption of the fluid the dissociation is what remains. As for now, there is no explanation for the genesis of CAS specialists should be more sensitive about its detection. Of note, a separate embryonic origin of the amnion and chorion has been challenged by recent studies in mice, postulating a common origin of single amnio chorionic fold with an early separation and a later fusion [22]. Hence, still many discrepancies exist in the literature regarding these fundamental processes and the situation in early human embryos is still unclear. Therefore, more data are needed to finally understand the physiological and pathophysiological mechanisms behind amnion chorion fusion and its genetic background.

The underestimation of the small sCAS in the routine praxis in the past could be explained by the used examination sonographic method and/or other subjective context reducing the motivation of the registration of the small sCAS before the planned genetic AC. In the German guidelines the AC will not be recommended at 15/0 SSW if the sCAS is present [6]. The small sCAS can be measured incorrectly, especially in patients with BMI > 40 and /

or using in appropriate, e.g., old technologies of ultrasound technic. The risk of premature delivery or miscarriage is two times higher per additional 1 mm of sCAS. The pathogenesis could be similar to the above mentioned. The risk of miscarriage is per se higher when diagnosed with chromosomal anomalies. Hence sCAS is associated with a higher risk for chromosomal anomalies the risk for miscarriage increases likewise. The study presented here did not determine a limit for CAS-measure regarding the increased risk of a pathological pregnancy or aneuploidy. The sCAS is suitable for use as a soft marker. Above a certain distance the sCAS can be a sonographic sign for chromosomal anomalies. The temporal limitation of CAS presentability has not been analysed. The distance of sCAS has been measured one time in our study before the AC, and the distance dynamic was not investigated. It could be an interesting point for forthcoming investigations. Also, it has not been determined weather another soft marker in combination with CAS can increase the risk of aneuploidy further. There were no procedure-related complications. So, the use of the 29-gauge needle is highly recommended for amniocentesis in case of CAS. The age as an influencing factor is quite inconsistent. Patients between 35 and 39 have per se a higher risk of fetal chromosomal anomalies or pathological pregnancy. But this age group was the most numerous. To examine the influence of the patients age as a disruptive factor more studies with bigger and especially more homogeneous proband groups are needed.

Nevertheless, the proven influence of CAS improves the decision support regarding a potential amniocentesis or NIPD. In case of persisting sCAS a determination of the fetal karyotype should be offered especially when other soft markers are found. The indication for amniocentesis can be hedged by measuring the sCAS. As it would be suitable to determine a certain limit another study with bigger population should be engineered. Quality assurance and safety advisory ensure a higher medicolegal security for medical participation. Every obstetrician should be capable of detecting and measuring a CAS and engineer a transfer to a malformation screening. For the pregnant women it is a chance of a higher level of surveillance of the pregnancy. A CAS is not necessarily accompanied by fetal aneuploidy or caused by pathological mechanisms. We found also cases with persisting CAS and normal karyotypes. After amniocentesis, iCAS to some degree can occur in around 25 % of cases, when it is searched for [23]. To our experience, it is even more common already before amniocentesis, but in most cases overlooked. Detailed studies on its occurrence prior to amniocentesis besides our study are lacking. Our definition of any CAS as diagnostic for the condition is supported by the above-mentioned study of Levine [23]. Additionally, we use a "pressure test" with the ultrasound transducer to see more detailed the movement of the amnion compared to the chorion in suspected cases, where the diagnosis is not obvious at first sight.

Indeed, the differences between the patients in this study compared to the former evaluated cohorts is, that they have more than 15 completed GW and thus, more amniotic fluid, but the fetal membranes are not developed as wide as it would be physiological. So, the most important question is, if the amniocentesis should be made in spite of the higher risk of a complication or if it should be postponed one week with the risk that the membranes even do not fuse until the next appointment, receiving the genetic result even later. This decision must be made with having regard on the indication for amniocentesis, the current ultrasound findings and the mother´s feelings about the two discussed possibilities. The further procedure has to be evaluated again for every patient. In former studies, a separation of chorion and amnion was found in five cases in two years of Appel man et al. [16] as well as in three cases in 13.000 routine scans [24]. These numbers show a big difference in comparison to our findings: 109 cases in three years. We expect the technical progress of the new ultrasound machines as the main reason that enables us now to diagnose the separated membranes much better than in some years ago. Appel man defined a non-fusion as "separation of the amnion and chorion beyond 14 completed gestational weeks with a distance of more than 10 mm between the two membranes" [16]. In this study, the median interstice between amnion and chorion was 1.27 mm (interquartile: 0.95 to 1.9 mm).

Thus, we cannot agree with Appel man's definition: Either the membranes are fused, or they are not. Consequently, would an interstice of 9.5 mm between the membranes after 14 weeks be defined as fusion? Even there is only a small space, amnion and chorion are not fused completely and so, it is not physiological and eventually associated with malformations.

Concerning this presumption, two aspects about the physiological development of fetal membranes, chromosomal abnormalities and amniocentesis stand in scientific discussion:

First aspect: Odibo postulated in 2008 a correlation between procedure-related complications after amniocentesis and chromosomal aberrations [18]. We can confirm this: the only spontaneous abortion in this study, twelve days after amniocentesis was a fetus with a Trisomy 21. So, it was not seen as procedure- but as malformation-associated. So, the aspect of CAD of a possible indicator of chromosomal abnormalities must also be considered and might be a rea-son to prefer non-invasive amniocentesis compared to free maternal DNA due to the limited diagnostic capabilities of the latter.

Second aspect: Appel man (1998=9) and Johnson (1999=19) postulated, that "delayed fusion of the amnion and chorion may be part of the developmental delay that occurs in these ab-normal pregnancies", meaning "delayed fusion of the amnion and chorion [is] related to fetal chromosome abnormalities" [16, 25]. The results of this current study confirm those findings: In a former study of our university center, we investigated procedure-related complication-rates after midtrimester-amniocentesis in 208 pregnancies with fused fetal membranes [11]. A pathologic karyotype was found in 10 cases, which means, that 4.8% of these women had an affected child. In contrast to these numbers, in this study, 16 fetuses in 109 amniocenteses were affected, meaning 14.7%. Consequently, we

found a 3.4-times higher risk for a pathologic karyotype in cases of a still remaining separation of the fetal membranes after 15 completed GW ($p<0.05$).

No child was born with an abnormal position of its feet, like it was found as complication for early amniocentesis in former studies [3]. The authors supposed the early gestational age at amniocentesis and the relatively high amount of missing amniotic fluid after the invasive procedure as the main reason of this phenomenon. In this series, only the development of the fetal membranes could be compared to those in early amniocentesis, not the quantity of amniotic fluid that increases from 71.4 mL in 13 weeks up to 191.2 mL in 16 GW, which means, that after an amniocentesis with 15mL in the 13th week 21.0% of the total amount of the amniotic fluid are missing, while its only 7.8% in the 16th week [26].

Conclusion

At least one or two amniocentesis-conditioned complications like membrane tenting, amniotic fluid leakage, amniotic fluid loss, vaginal bleeding, (iatrogenic) preterm premature rupture of membranes (iPPROM) and total fetal loss could have been expected: But all 109 invasive prenatal diagnoses had a positive pregnancy-, maternal- and fetal outcome without any of the examined complication at the time of 7 days. 28 days after the amniocentesis, apart from 13 pregnancies that were terminated as well as two with a poor outcome in consequence of a Trisomy 21, all fetuses were still alive. 89 patients delivered live born children, which finally means, that amniocenteses in this series were successful in 100% of all cases. Reducing the expected complication-rate significantly, the much smaller diameter and the atraumatic sharpened tip of the 29G-pencil point needle as well as the 36-times smaller leakage in the amniotic membranes are seen as the main reasons for this success [12].

Shortcomings of our study are the small group: 145 women were included in this study which doesn´t allow us to postulate the complication-rate of nearly zero per cent as a definite rate. Besides it is not a prospective-randomized trial, which are very sparse in the topic of invasive prenatal diagnosis and don´t lead to any practically important conclusions so far [27]. Furthermore, this was the first study about mid-trimester amniocentesis with separation of the fetal membranes, and we had no data, to which our results could be compared. Nevertheless, those limited experiences, the result of this study offers a save technique for women with a high-risk pregnancy asking for invasive prenatal diagnosis. Invasive prenatal diagnosis nowadays is becoming a less frequent procedure due to non-invasive prenatal testing (NIPT) via cell-free DNA [28]. If invasive procedures like amniocentesis is compared to NIPT, it should also be taken into account to compare it with the method with the lowest risk of adverse outcome which to our opinion and experience is the atraumatic amniocentesis with the 29 Gauge needle. It should also be stressed, that nearly a third of abnormalities after first-trimester screening are different than expected and would go undetected with NIPT [29]. The CAS might also be an indication for invasive testing to rule out chromosomal abnormalities beside aneuploidies.

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