

**The disturbed perinatal adaptation and its late outcomes in term
and preterm neonates**

Ph.D. Thesis

Vida Gabriella M.D.

**Departement of Obstetrics and Gynecology, Faculty of Medicine, Univesity
of Pécs**

Univesity of Pécs, Faculty of Health Sciences, Ph.D.School



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Introduction

In 1970 the chances for premature infants of surviving were very small in Hungary. For infants between 1000-1500 grams chances were about 30%, and for infants under 1000 grams chances were even as low as 0-1%. In 1980, after the Perinatal and Intensive Center had been established, we could see distinct improvements in both groups. 10% of the infants under 1000 grams and 45% of the infants between 1000-1500 grams survived the neonatal period. In 1998, WHO suggested the lowest limit of natality to be 24 weeks. After that, despite the decrease of births, the number of light weight premature infants has increased. After 2000, 75-80% survived in both groups, but 10-20% of these premature infants live with a serious neurological deficit. Realizing the early impairments and using rehabilitation in time, chances of a qualitative life can be improved.

Nitrogen monoxide is an important neurotransmitter and a vasoactive metabolite. In 1990s researchers noticed the nitrogen oxide synthases (NOS), which is an enzyme system suppressor asymmetric dimethylarginine (ADMA). Several cardiovascular diseases like hypertension and atherosclerosis, ischaemic heart disease, plus chronic kidney- and hepatic diseases, diabetes mellitus, hypercholesterolemia and even smoking can cause high ADMA level. Pregnant women have a very low ADMA level compared to non pregnant (healthy) women. Still we haven't got enough data yet about the ADMA concentration in the umbilical cord and of the peripheral blood of the neonate.

Aims

1. We would like to know whether we can show any changes in the ADMA/SDMA/L-arginine system during adaptation.
2. We want to demonstrate the effect of the stress caused by spontaneous birth and cesarean section at giving birth with the release of the examined vasoactive agent in case of a mature neonate after normal adaptation.
3. We want to follow up the differences between the adaptation of premature infants and mature neonates in ADMA/SDMA/L-arginine system.
4. According to the „fetal programming” hypothesis, it is likely that not only the dysmature but also the immature infants can have such a metabolism process which can cause chronic diseases like cardiovascular, renal aberrancy, diabetes mellitus type 2 or hyperlipidemia in their adulthood. Can the cardiovascular problems which arise in adulthood be predicted by the parameters we check?
5. Yearly we treat about one-hundred neonates under 1500 grams. We nurse most of them until we let them go home. Tests are done during the first 3-4 months in our hospital. Studying the test results gives us the possibility to understand the reliability of the prognoses to predict impairments at a later stage in life.
6. Every neonatal / perinatal intensive center should study its own results and not only know the mortality rates but also know the chances for a good life quality for those who survive. This way parents can be informed correctly. Studying the background of neonates adaptation could help improve surviving chances for infants in low gestational groups. The aim is to reach a survival rate of 70% or more in each gestational age.
7. Beyond improving the surviving chances it's very important to emphasize the early screening of impairments, the predictive function of early screening and to begin the early rehabilitation to improve also the quality of life.

Patients and methods

We attended 1499 premature infants in the Neonatal Intensive Center of the Obstetrical and Gynecological Clinic of PTE OEKK ÁOK between 1st January 2000 and 31st December 2004. This means 20% of the infants were premature, which is much higher than the national average. This can be explained by the fact that our clinic is a regional center and attends pregnant women with a higher risk factor. Our clinic operates in 3 counties. An important fact is that since 1998 the number of premature births has increased in our clinic although the number of the absolute births has decreased:

1992:	13,1%
1998:	16,8%
2000-2004:	20%

In retrospective we reviewed the documentation of 272 infants born before the 29th week. Among them 3.2% (48/272) were born before the 25th week (2.chart).

We didn't mention those babies which had to be delivered unnaturally and which had such a birth defect that they were unable to live (10 premature infants).

We summarized the results by gestational weeks of the skull ultrasonics, retinopathia praematurorum screening, auditive screening with otoacusticus emissio, chronic pneumopathy. After it we systematized the neurological status of the premature infants who were sent to the neurological development aftercare. We also reviewed the data's of ophthalmological, audiological aftercare.

We examined the Asymmetric dimethylarginine, symmetric dimethylarginine, L-arginine serum levels on 10 mature infants born with spontaneous vaginal birth and on 10 mature infants born with cesarean section plus on 20 premature infants between 1st July 2005 and 31st November 2006.

The gestational age of mature infants born by spontaneous vaginal birth were 39,4±0,97 week, their birth weights were 3330±405 gram. The gestational age of infants born by cesarean section were 39,5±1,5 week and their birth weights were 3526±516 gram. The gestational age of the premature infants were 26,7±1,6 week (range:24-29 week), their birth weights were 919,5±235 gram (range: 480 -1280 gram).

The mothers didn't suffer from any chronic or other disease related with pregnancy. Women who gave birth to mature infants didn't get tocolytic therapy, but those mothers who gave birth to premature infants got beta-mimetic tocolysis before the delivery (like is written in the protocol). In case of mature birth the reason of cesarean section was always the mother (spatial disproportion, previous cesarean section in anamnesis).

We sampled arterial and venous blood plasma from the neonatal umbilical cord on the side of the placenta right after attending it.

On the second postnatal day we examined the venous serums we obtained by routine blood tests.

In case of premature infants we also used serums of routine blood tests we had weekly. The samples were taken in EDTA tubes (pipes) and after 15 minutes of centrifuging we stored them on -20°C degrees until processing. The measurements were made in the Pharmacological Institution of Magdeburg University Clinic.

Results

1. Asymmetrical dimethylarginine, symmetrical dimethylarginine, L- arginine examinations on mature neonates

In case of mature neonates born by vaginal delivery we can observe veno-arterious (V-A) differences and significant postnatal reduction in L-arginine levels. In case of cesarean section there is no veno-arterious difference, but the postnatal reduction is still significant. We can't show significant differences considering birth method, either in umbilical values or in the range of postnatal reduction.

The ADMA concentrations showed significant veno-arterious differences and also significant postnatal reduction in case of vaginal delivery. In case of cesarean section there is no A-V difference but there is significant postnatal reduction. We examined the ADMA serum levels and haven't found statistical differences neither in umbilical values nor postnatal considering birth methods. We can show borderline significance (signification) ($p=0,054$) between the umbilical arterious serums taken after spontaneous delivery and after cesarean section.

In SDMA plasma concentration of mature neonates there is no veno-arterious difference and no postnatal reduction neither in case of those infants who were born by cesarean section nor those infants who were born by spontaneous birth. After sectio caesarea we measured significantly higher SDMA levels than after spontaneous delivery.

2. Asymmetrical dimethylarginine, symmetrical dimethylarginine, L- arginine examinations on premature infant

In the umbilical artery the L-arginine level is lower ($41,01 \pm 19,11 \mu\text{mol/l}$), than in the umbilical veins ($47,84 \pm 23,60 \mu\text{mol/l}$, $p < 0,05$), and on the second postnatal day according to the umbilical serums we can see notable decrease ($26,67 \pm 12,14 \mu\text{mol/l}$, $p < 0,001$). The L- arginine level increases remarkably from the 2nd day until the 3rd postnatal week ($26,67 \pm 12,14 \rightarrow 83,66 \pm 41,10 \mu\text{mol/l}$, $p < 0,001$).

There is no significant difference between the values measured at the same time in case of premature boys and girls. Nor is there significant difference between the values measured at the same time in case of premature infants born by cesarean section or by spontaneous vaginal birth.

In case of ADMA there is no veno-arterious difference in the umbilical arteries ($0,96 \pm 0,29 \mu\text{mol/l}$, vena umbilicalis: $0,94 \pm 0,30 \mu\text{mol/l}$), but we can observe a significant decrease on the 2nd postnatal day ($0,64 \pm 0,25 \mu\text{mol/l}$, $p < 0,001$). Similarly to L- arginin from the 2nd day until the 21st day we can measure a steady increase ($0,66 \pm 0,16 \rightarrow 0,98 \pm 0,23 \mu\text{mol/l}$, $p < 0,001$).

Because of the low amount of cases we can not draw a correct conclusion, but in the umbilical artery the ADMA level is higher when there is an intrauterine infection, than in case of premature infants who did not get intrauterine infection ($p = 0,08$).

In the SDMA levels we can not see umbilical veno-arterious difference (umbilical artery: $1,59 \pm 0,39 \mu\text{mol/l}$, umbilical venous: $1,63 \pm 0,40 \mu\text{mol/l}$), neither can we observe postnatal decrease by the 2nd day ($1,72 \pm 0,51 \mu\text{mol/l}$). From the 3rd week (after the stagnation by that time) to the 4th week we can see a significant decrease ($1,78 \pm 0,33 \rightarrow 1,50 \pm 0,43 \mu\text{mol/l}$, $p < 0,05$).

We examined the total effect of sex, the method of birth, the gestational age, the birth weight, dopamine demand, using a ventilator, Apgar numbers, the age of the mother, oxygen demand, BPD caused steroid demand, intrauterine or later infections on the change of L-arginine, ADMA, SDMA serum level or on arg/ADMA rates with multi-variant linear regression analysis. With the progress (development) of the postnatal age we can observe an increase on the L-arginine level ($p < 0,001$) and on the ADMA levels ($p < 0,001$). Other factors that we examined have effects on the L-arginine. The ADMA has a negative correlation with the gestational age ($p = 0,001$), with the 3rd day dopamine demand ($p = 0,014$), plus with the late, after the first week occurring infections ($p = 0,037$). A higher birth weight results in higher L-

arginine ($p = 0,052$) and ADMA ($p = 0,002$) concentrations. The next table shows the values in details.

	L--Arg (p)	ADMA (p)	SDMA (p)	L-Arg/ADMA (p)
Gestational age	-12.8 (0.194)	-0.17 (0.007)	-0.19 (0.104)	3.586 (0.715)
Birth weight (100g)	85.13 (0.052)	0.863 (0.002)	0.553 (0.286)	1.337 (0.756)
Apgar 1 min	-9.62 (0.197)	-0.07 (0.101)	0.154 (0.086)	-5.10 (0.492)
Apgar 5 min	10.27 (0.330)	0.034 (0.611)	-0.10 (0.411)	8.518 (0.418)
Female gender	12.91 (0.243)	0.048 (0.493)	-0.15 (0.244)	10.78 (0.328)
Maternal age	1.826 (0.209)	0.016 (0.081)	-0.00 (0.895)	1.029 (0.476)
Cesarean section	-0.51 (0.980)	0.006 (0.959)	0.303 (0.232)	-10.3 (0.623)
Ventilation	1.275 (0.561)	0.001 (0.887)	0.004 (0.862)	1.794 (0.414)
Oxygen-need	-7.83 (0.640)	-0.11 (0.284)	0.276 (0.172)	-7.40 (0.658)
Steroid-need	-9.03 (0.588)	-0.12 (0.244)	-0.24 (0.216)	1.365 (0.934)
Early infection	-11.8 (0.238)	-0.11 (0.088)	0.168 (0.162)	-5.76 (0.562)
Late infection	-13.4 (0.481)	-0.25 (0.037)	-0.31 (0.169)	14.22 (0.455)
Dopamine-need at 3 rd day	-13.1 (0.365)	-0.23 (0.014)	0.330 (0.060)	2.189 (0.879)
Dopamine-need at 7 th day	8.999 (0.686)	-0.03 (0.780)	0.571 (0.035)	20.46 (0.359)
Postnatal weeks	15.13 (<0.001)	0.118 (<0.001)	-0.02 (0.339)	7.803 (0.005)

3. Comparing the examination results of mature and premature infants

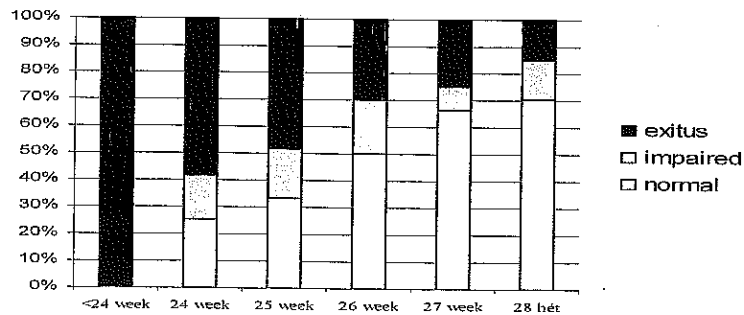
The determined values in the umbilical vessels of premature infants and the first postnatal values are significantly lower than the serum concentrations detected at a similar time of mature infants and mature infants born by cesarean section.

There is no significant aberration in the umbilical ADMA serum levels and the first postnatal values of infants born by spontaneous vaginal birth and mature infants born by cesarean section or premature infants.

We measured much higher SDMA levels at those infants who were born by cesarean section than in case of infants born by vaginal birth or those who are premature. Those mature neonatals who were born spontaneously have much lower postnatal first values than those infants who were born by cesarean section or those who are premature.

4. Results achieved by screening (brain ultrasound, hearing, vision)

Between 1st January 2000 and 31st December 2004 there were five neonatals who were born in the 24th gestational week. The mortification was 100% (because of cerebral bleed or pneumorrhagia) On the 24th week 41.9% of infants survived and 25% survived without any damage. We can see step improvements from the 26th week, 70% of the infants survives and 54% survives without any permanent damage. The next figure shows the values in details:



4/1. The predictive value and significance of the early aberrancies

We divided the early damages in weekly dissociation by retinopathy, serious intraventricular hemorrhage, bronchopulmonalis dysplasia. We emphasize, that the serious retinopathy and the bronchopulmonalis dysplasia are the most common among the premature infants born in the 25th week but the IVH and PVL are the most common in the 26th and 27th gestational week. By the consolidated results between the 24th -28th gestational week the bronchopulmonalis dysplasia occurs in 24%, intraventricular hemorrhage and cystic periventricularis in 6,8%, ROP which needs an operation occurs in 14,2%. These data are in line with national and international data.

4/2. The postnatal by the 12. months adjusted , permanently remaining damages

The late tracking is not always possible because of the social background of the parents, 79% of the patients appeared at aftercare. More than 60% of the infants born after the 25th gestational week seemed to be neurologically healthy. Among the examined patients (24-28 gestational week) 5,7% are blind or have serious problems with their seeing, 3,4% needs a hearing aid and 11% are mentally retarded or handicapped. Eight children live at least with two different damages.

Discussion

It's known that the ADMA level of pregnant women is lower than that of women who are not pregnant. Compared to the mother's serum we measured quadruple ADMA levels in the umbilical cord serums. This is a paradoxical result, because it is known that the vascular resistance of the foetoplacental circulation is low in contempt of the really high ADMA concentrations. The vascular resistance in the foetoplacental circulation is probably upheld by a difficult feed-back mechanism (NO-DDAH-ADMA-NOS), for example by the S nitrosylation.

In the umbilical cord blood we measured raised ADMA and SDMA levels. This can have three reasons, either the oversupply of precursor substance, inadequate function of the enzymes which take part in metabolism or reduced diuresis via the kidneys. The increased protein metabolism in the foetoplacental circulation is extremely intensive, besides this the enzymatic and also the renal elimination reduced according to the supply. The DDAH activity is low during the fetal life, but after birth it increases rapidly, the diuresis also rises with the maturity process of the renal function. In the umbilical veins (placental side) the measured values are higher than in the umbilical artery (fetal side), which also refers to the fact that the ADMA compound is mainly produced by the placental endothelium. The enzymatic processes which start after birth, result in a fast decrease of the ADMA level in the case of mature well adapting neonates. The relevantly raised SDMA levels, the absence of the umbilical arteriovenous difference and the absence of the drop on the second postnatal day can be explained by immature kidney function on the first days, because the SDMA can only leave the body renally.

The raising of L-arginine and ADMA levels during birth can have a role in supporting the normal blood pressure and the adequate cardio-cerebro-vascular circulation in the course of delivery.

It is known that among premature infants hypertension or other cardiovascular alterations can occur during their puberty and their adulthood. ADMA as an early marker can indicate endothelium dysfunction. First Barker documented this, that is why the origin of the metabolic syndrome foetalis, diabetes mellitus type 2 and chronic cardiovascular diseases are called Barker hypothesis.

The presence of the oxidative stress and its harmful effect is known among very low weighted premature infants (ROP, BPD). Experiments have shown that the oxidative stress raises the ADMA levels via the hindrance of the DDAH enzyme system and thus increases the activity of the methyltransferase. This can also explain the raised ADMA and L-arg/ADMA ratio in the first month in the case of premature infants who are under 1250 grammes and in need of mechanical breathing support and permanent oxygen supplement.

After birth the hormone systems of the neonates activate, their increased function is known, as a result of this the activity of ADMA and also of the renin-aldosterone-angiotensin system (RAAS) reaches its maximum (point) at around the 3rd -4th week. We can show the coherency between the L-arginine- ADMA system and the RAAS with knowing that the ADMA system can be influenced by medications which hinder the angiotensin converting enzyme. We assume the connection of these two systems in upholding the normal blood vessel tone or in case of its delay the development of endothel dysfunction.

The purpose of prenatal care is to reduce the number of premature births. Within the framework of progressive medical attendance in case of threatening premature birth it is reasonable to hospitalize the mother into such an institution where not just the pregnant woman, but also the immature neonate can get a high level of attendance in the Neonatal Intensive Center. The intrauterine transport is advantageous for the fetus and for the mother as well. Nowadays the real challenge is not just keeping the micro-premature infants alive, but creating a better quality of life.

The purpose of screening is to recognize early impairments in time and give them up to date treatment. Vohr and his colleagues examined a lot of patients. According to these examinations we can say that after serious cerebral bleeding the following examinations which were done at the age of five don't show any neurological aberration in 60% of cases.

In the premature infants population the increase of the number of blind children is caused by the high retinal immaturity. The early treatment significantly reduce the rate of people with vision impairment.

The audiological training, when started in time, or wearing a hearing aid if it's necessary can help the normal contact-making with the environment and the adequate speech process development.

To sum up we can state that the life prospects of the really low gestational aged premature infants are gradually getting better with the progress of the gestational age. By international surveys in those institutions where there are more patients, the chance of life for infants under 1500gram and their quality of life is significantly better than in smaller centers

We find it important that the attendance and treatment of premature infants should happen in such intensive centers where the required technical equipment is available, where the number of yearly patients provide opportunity for the staff to get into a professional routine as well as high level professional courses and application of the academic results. We emphasize that the birth of really immature micro-premature infants should possibly happen in such a maternity institution which has a neonatal intensive center to avoid the trauma of transportation and acute care. It is the only way to improve the survival and achieve a higher quality of life.

The future life prospects and social integration of surviving premature infants depend on such permanent impairments as reduced vision, hearing impairment, somatomental developmental disorder and chronic pneumopathy. These permanent impairments can be well predicted with the examination of the early skull ultrasound, ophthalmological screening and with the measurement of the otoacoustic emissions. This makes early treatment possible.

Summary , recommendations

The ADMA serum concentration values measured in the umbilical cord blood were examined and are accessible in international literature in just four cases including our communique as well [53,55,56,61]. Data about the umbilical artery are even harder to find, although the levels of the umbilical artery serums reflect the foetal side. In the umbilical blood vessels, plus with the measurements we made in the early postnatal period we provided raw data for the adaptation of normal neonates.

It can be stated that in case of mature or premature neonates there is no significant difference in serum ADMA/L-arginine concentrations either in case of spontaneous, per vias naturales

birth or birth by cesarean section. We can measure significantly raised ADMA/L-arginine concentrations in the umbilical blood vessels and the postnatal reduction can be shown statistically by the third day. The serum SDMA levels are higher in case of mature neonates born by cesarean section than in case of mature infants born by spontaneous, vaginal way or in cases of premature infants.

The ADMA, SDMA and L-arginine results of the really low weighted premature infants adapted in a pathological way show the L-arginine-NO system's increased activity during the first three weeks. This can be explained by the need for extra oxygen, late infections, maturation of BPD and the mechanical breathing which is necessary in this gestational age. After the reduction in the first three days we can see rising L-arginine, ADMA levels, then in the fourth week this tendency stops, showing the improvement of the neonate's condition. SDMA levels are just slowly decreasing into the normal domain, which refers to the fact that the immature premature infant's kidneys are functioning.

The permanently high serum ADMA values, like the markers and mediators of the endothel dysfunction reflect the state of the cardiovascular system in case of premature infants, so probably they predict the cardiovascular diseases which may occur in adulthood .

In our Clinic we are among the firsts in the country to regularly make skull ultrasound screenings on neonates and premature infants since 1991. We have a good relationship with the developmental neurologist and the neurosurgeon consultant colleagues. Following the concerned premature infants or neonates in a team gave good results in the later life quality.

After processing of our mortification results we are able to give recommendations to our colleagues in the attendance-screening team and our department's colleagues to give the neonates a better qualified attendance. It can be stated that among doctors and ancillary workers participated in premature infants' attendance, communication is one of the most effective healing methods.

In the interest of the improvement of qualitative survival we were among the first in the country to initiate skull ultrasound screenings and otocoustic emissions as a hearing screening examination. The screening of retinopathy and its operation happens as a routine in the clinic, so we can avoid the trauma of transportation and reduce the number of infants with a serious visual impairment. Habilitation training was already started early in the clinic, and this provides a good foundation in the development of movement. To teach the basic things gives opportunity and confidence to the parents. When they get home from the hospital they can start the early development on time.

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tudományos munka elkészésének, végzésének, befejezésének eleinte átláthatatlannak látszó

tervezésében. Biztatott az olykor megvalósíthatatlannak tűnő feladatok megoldására. Azt,

hogy még neonatológusként dolgozom, némi kitérő után is, jöveszi neki köszönhetem.

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