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ARTICLE

Major decrease in the incidence of trisomy 21 at birth in south Belgium: mass impact of triple test?

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In South Belgium (Wallonia), the 'triple test' was introduced in 1990–1991, and is nowadays a widely accepted screening method for assessment of trisomy 21 risk in pregnancy. The 'triple test' is not regulated and can be freely performed by any biomedical lab, making epidemiological data unavailable. By contrast, cytogenetic investigations are limited to a few genetic centres, and accurate statistics can be easily built from their files. During the period 1984–1989, a total of 244 trisomy 21 (1/876 pregnancies) were diagnosed in the Genetic Centres of Liège and Loverval, 42 (17%) of them prenatally. During the period 1993–1998, 294 trisomy 21 (1/704 pregnancies) were observed, 165 (56%) of which prenatally, and more than 90% of affected pregnancies were terminated. Even after correction for late foetal loss of trisomic foetuses, the difference is highly significant, and corresponds to a theoretical shift in the incidence of trisomy 21 at birth from 1/794 to 1/1606. As no remarkable progress occurred in other non-invasive prenatal screening procedures or general health care policies in Belgium, the most reasonable explanation is the use on a large scale of triple test by pregnant women, and the election of termination for most affected pregnancies. *European Journal of Human Genetics* (2001) **9**, 1–4.

Keywords: trisomy 21 incidence; triple test; prenatal diagnosis; epidemiology

Introduction

In many countries, prenatal screening for Down syndrome risk is now well established and almost a routine procedure. Commercial kits have been developed and the test is systematically offered to cohorts of pregnant women. During the second trimester, different maternal serum markers have been proposed: human chorionic gonadotrophin (HCG) or its variants (free β HCG, core HCG), α-foetoprotein (AFP) and unconjugated oestriol, these three markers forming the 'classic triple test'. During the first trimester, PAPP-A and free β HCG are proposed; the latter can be combined with the study of nuchal translucency. The triple test is not diagnostic: it gives an evaluation of the risk. If the risk is higher than a predetermined cut-off (varying from 1/250 to 1/350 at time of birth), an invasive prenatal diagnosis, usually amniocentesis, is suggested. If the results show an affected foetus and the couple agrees, termination may be elected. Therefore, the birth prevalence of trisomy 21 should tend to decrease (Spencer and Carpenter[1]).

Belgium is a federal kingdom, composed of three administrative regions with distinct governments (Flanders, Wallonia, and Brussels). Wallonia, the southern, French-speaking part of Belgium counts about 3.5 million inhabitants. Triple test was not performed in any laboratory in Belgium before 1 January 1990. It was introduced by our team in Wallonia in 1990, and was rapidly proposed to a large proportion of the pregnant women in South Belgium. The Genetic Centre of Liège offers the triple test using a locally developed dried blood technique (Verloes *et al*[2]) which is also commercially available (Gamma®, Liège, Belgium). We perform about 13 000 tests per year (Verloes *et al*[3]), ie roughly in 1/3 of all pregnancies in Wallonia. In our laboratory, about 15% of the tests are performed during the first trimester of pregnancy. Cut-off risk has been set at 1/250 at term. However, between 1/250 and 1/350 gynaecologists are invited to decide whether they advise amniocentesis or not. In Belgium, the practice of medicine is unrestricted and the triple test is not regulated. Hence, screening for trisomy 21 in maternal serum can be

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performed – and in practice is performed – by many public and private laboratories, without any particular control or restriction, and has been rapidly accepted as a routine procedure by most obstetricians. It is therefore impossible to know how many women actually undergo the test in Wallonia, which kits are used, when the test is requested (first or second trimester) and which cut-offs are used. Nevertheless, based on our local experience, we estimate that at least between two-thirds and three-quarters of pregnancies are screened.

In contrast to the free and widespread practice of triple test, cytogenetic investigations in Belgium are restricted to eight Human Genetic Centres, linked to medical schools, and recognised by federal decree. No private laboratory undertakes cytogenetic investigations. The centres perform all genetic analyses. As no diagnosis of trisomy 21 on any material (blood, amniotic fluid, chorionic villi, abortion material) is made outside the genetic centres, it is very easy to collect accurate data concerning all chromosome anomalies detected in Belgium during a given period, as the whole population is surveyed. In South Belgium, two genetic centres exists, in Liège and Loverval. Almost 99% of all births take place in hospital. A survey of maternities by our two centres in collaboration has shown that we roughly cover together 90% of all births in Wallonia. Hence the exact number of trisomies, detected either pre- or post-natally is precisely known.

The aim of the present study was to determine whether the use of large-scale triple test screening in South Belgium had a measurable impact on the epidemiology of trisomy 21, by comparing the 6-year period preceding the implementation of the test with the 6-year period following it.

Although Wallonia is not an independent country, but a federal unit, the present study is closer to a national survey than a regional survey, because the 'borders' between the federal areas are really socially and culturally significant and allow population-based data analysis.

Material and methods

Data on trisomy 21 diagnoses of mothers living in Wallonia and children born in Wallonia, were collected in the genetic centres of Liège and Loverval.

Official statistics are available in Belgium for the whole country and separately for the three regions through the Institut National de Statistiques (INS). The actual total number of births is known up to 1995 and estimates are available for 1996–1998. Data have been published up to 1992 concerning the mother's age when giving birth (INS, 1996).[4] Note that, because official statistical tables are continuously updated and edited, the number of pregnancies extracted from the INS 1996 reports (by maternal age), and those obtained in December 1999 (INS, unpublished data) and used in Table 3, are slightly different from each other.

Since 1971, most pregnancies and births in Wallonia have been supervised for chromosome analysis by the genetic centres of Liège and Loverval and annual data and mode of diagnosis are available and regularly updated (Koulischer and Gillerot;[5] Koulischer *et al*[5]).

Data on trisomy 21 gathered in our two laboratories have been split into two groups for analysis. The period from 1984 to 1989 (PRE) represents the 'pre-triple test era', when most amniocenteses were undertaken for maternal age or ultrasound anomalies. The triple test was implemented in South Belgium in 1990 and rapidly gained wide acceptance. Based on our own recruitment, the period 1990–1992 can be considered as the 'transition era'. The period from 1993 to 1998 inclusive is the post 'triple test era' (POST), during which the triple test has been widely offered in South Belgium and can be considered as the major modification in prenatal diagnosis strategies.

Because spontaneous foetal loss occurs during the second and third trimesters of pregnancy, the number of trisomic foetuses detected by CVS and amniocentesis is higher than the actual number of potential trisomic newbirths. Schreinemachers *et al*[7] calculated this spontaneous loss to represent 1/5 of the prenatally detected cases.

All statistics have been assembled using standard procedures, with the Statistica Package version 6 (Statsoft®, Tulsa, USA) running on a PC.

Results

Based on our previous data on incidence of trisomy 21[6] and on the population data from the INS, expected incidences at birth without any prenatal diagnosis have been computed (Table 1) for 1987 (mid of the PRE era) and 1992 (last available data by maternal age), taking account of the maternal ages. Those incidences are close to 47 and 54, respectively.

Diagnoses of trisomy 21 made on blood, amniotic fluid cells (AF), or chorionic villi (CVS) between 1984 and 1998 are shown in Table 2 in absolute levels (using raw data and data corrected following Schreinemachers[7]) and in Figure 1 in relative proportions of pre- and postnatal diagnosed cases (using corrected values). Yearly fluctuations in absolute numbers are noteworthy: from 23 to 63 cases/year. The mean number of cases/year between 1984 and 1998 is 44.6, (SD:

Table 1 Anticipated trisomy (T21) births in South Belgium, based on demographic data from 1987 and 1992

Maternal age	*<20*	*20–24*	*25–29*	*30–34*	*35–39*	*40+*	*Total*
Incidence of T21	0.47	0.68	0.87	1.29	4.40	14.68	
No. births, 1987	1934	11 385	15 584	7674	2201	343	39 126
No. births, 1992	1688	9 653	16 434	9740	3131	454	41 108
Anticipated T21, 1987	0.90	7.74	13.56	9.90	9.68	5.03	46.81
Anticipated T21, 1992	0.79	6.56	14.29	12.56	13.77	6.66	54.63

Table 2 Trisomy 21 (T21) diagnosed from 1984 to 1998 at birth, in amniotic fluid (AF) and chorionic villi samples (CVS) and percentage postnatally detected

Year	*Births*	*T21 newborns*	*T21 by AF*	*T21 by CVS*	*Total prenatal*	*Corrected prenatal*	*Total T21*	*Corrected total*	*Anticipated number**	*Proportion postnatal*	*Corrected proportion postnatal*
1984	38 038	20	6	0	6	4.8	26	24.8		0.769	0.806
1985	38 356	34	1	1	2	1.6	36	35.6		0.944	0.955
1986	39 511	30	4	1	5	4	35	34		0.857	0.882
1987	39 628	34	8	0	8	6.4	42	40.4	42	0.810	0.842
1988	40 802	48	11	0	11	8.8	59	56.8		0.814	0.845
1989	41 072	36	10	0	10	8	46	44		0.783	0.818
1990	41 210	27	11	2	13	10.4	40	37.4		0.675	0.722
1991	42 189	31	6	2	8	6.4	39	37.4		0.795	0.829
1992	41 177	34	17	1	18	14.4	52	48.4	49	0.654	0.702
1993	39 397	22	17	4	21	16.8	43	38.8		0.512	0.567
1994	37 905	13	32	0	32	25.6	45	38.6		0.289	0.337
1995	37 586	21	30	0	30	24	51	45		0.412	0.467
1996	38 497	26	20	0	20	16	46	42		0.565	0.619
1997	38 584	18	28	0	28	22.4	46	40.4		0.391	0.446
1998	38 326	29	34	0	34	27.2	63	56.2		0.460	0.516

Raw data and values corrected for spontaneous fetal death (–20%) are given. As a test for completion of the data collection, the actual number of trisomies observed in 1987 and 1992 is compared with the anticipated number*, which represents 90% of the number of trisomies computed in Table 1.

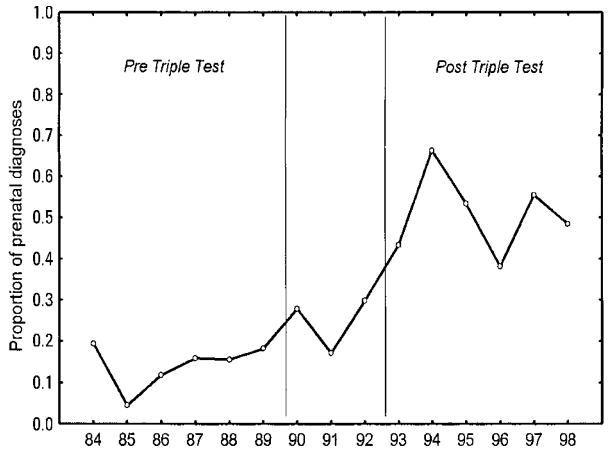

Figure 1 Relative frequencies of prenatal *vs* postnatal diagnosis of trisomy 21 in South Belgium, 1984–1998 (corrected values).

9.3–95% confidence interval : 39.4–49.8). Accounting for spontaneous loss, the expected number of trisomic newborns would have been 41.3/year. (SD: 8.2–95% CI: 36.8–45.9) in the interval 1984–1998. For 1987 and 1992, the observed values are not significantly different from the values extrapolated from maternal ages, thus confirming our high ascertainment ratio. Interestingly, most prenatally diagnosed trisomies have been terminated. For some pregnancies, the outcome was not available, but on a total of 207 prenatal diagnoses, we are aware of more than 190 abortions. We estimate with confidence that 90–95% of prenatally diagnosed trisomies are aborted.

Regression of the proportion of prenatally diagnosed cases (corrected values) on decimal year for the full set and, separately, for the PRE and POST periods, show that the slope is significantly different from 0 for the whole set ($P < 0.01$), whereas is not significantly different from 0 (Table 3) for each subset.

Table 4 shows basic statistics and comparisons between the PRE and POST periods. There is a trend to an increase in the total number of trisomies 21, although the difference in mean number of trisomies is not significant when PRE and POST are compared. A highly significant difference is observed between the two periods when the absolute numbers or the proportion of prenatal diagnoses are compared. Table 4 also shows incidence of trisomy 21 (with or without correction) and its theoretical incidence at birth (assuming systematic termination) in Wallonia, for the PRE and POST periods.

Discussion

When an ultrasound scan is used to estimate gestational age the detection rate for a 5% false-positive rate is estimated to be 59% using the double test (AFP and hCG), 69% using the triple test (AFP, hCG, uE3) (Wald *et al*,[8]). This goal has been almost achieved 'spontaneously' in Wallonia, where no systematic screening has ever been organised, and different techniques are used. In our population, the mean (corrected) incidence of trisomy 21 was 1/906 and 1/794 for the PRE and

Table 3 Decline and its limits in the regression of the proportion of prenally diagnosed foetuses (corrected values) to the year (in decimals) for the whole period, the PRE period and the POST period

	Slope	*Inferior limit (P=0.99)*	*Inferior limit (P=0.95)*	*Superior limit (P=0.95)*	*Superior limit (P=0.99)*
Full set	0.0355	0.0171	0.0223	0.0487	0.0539
PRE	0.0089	–0.0545	–0.0294	0.0472	0.0724
POST	–0.0064	–0.1276	–0.0795	0.0667	0.1148

Table 4 Comparison of the detection rate of T21 in the periods 1984–1989 and 1993–1998

		PRE (1984–1989)	*POST (1993–1998)*	P *level*
Number of T21	Raw	244	294	
	Corrected	235.6	261	
	Not detected	202	129	
Population[a]		213 666	207 265	
Detection rate	Raw	17%	56%	<0.0001
	Corrected	14%	50.6%	<0.0001
Total/year ± SD	Raw	40.7 ± 11.3	49 ±7.3	0.16
	Corrected	39.3 ± 10.8	43.5 ±6.7	0.43
Prenatal/year ± SD	Raw	7 ±3.3	27.5 ± 5.8	0.00002
	Corrected	5.6 ±2.7	22 ±4.6	0.00002
Incidence	Raw	$11.4\ 10^{-4}$ (1/876)	$14.2\ 10^{-4}$ (1/704)	
	Corrected	$11\ 10^{-4}$ (1/906)	$12.6\ 10^{-4}$ (1/794)	
	Not detected	9.410^{-4} (1/1058)	$6.2\ 10^{-4}$ (1/1606)	

Data are ± 1 SD. Raw and corrected values (which reduce the actual number of prenatally diagnosed cases) are included. (*P* levels are based on the *t* values for each comparison. [a]total number of births corresponds to 90% of the total number of births in Wallonia (see Introduction).

the POST periods, respectively. This increase appears linked to an increased mean maternal age, as observed between 1987 and 1992. The observed incidences of trisomy diagnosed at birth during the same periods are 1/1058 and 1/1606, respectively, corresponding to a shift of 14% to 50.6% in the detection rate. Figure 1shows that the decline is not the result of a continuous trend, but rather that the PRE and POST periods had different but stable detection rates. As no remarkable progress occurred in other non-invasive prenatal screening procedures (e.g. ultrasound) or general health care policies in Belgium between 1990 and 1992, the most reasonable explanation is the use on a large scale of the triple test followed by amniocentesis of pregnant women, and election to terminate most affected pregnancies. Interestingly, in our region, CVS which was common practice in the late 1980s has gradually disappeared as a routine cytogenetic procedure, and is now restricted to specific indications with high recurrence risks (parental translocations, biochemical or molecular diagnoses of Mendelian disorders).

A remarkable point is the huge variation in the yearly number of trisomies. The most likely hypothesis is that these variations are stochastic fluctuations associated with the small numbers involved. At least, it shows the usefulness of using the data obtained during a short period of time, and makes the estimation of the proportion of prenatal diagnosis a better indicator that the absolute number of cases, which depend on demographic fluctuations, as illustrated in Table 1, which shows that, in a short lapse of time (5 years), a significant increase in the older age group has occurred.

It is to be expected that the use of new serum markers and the routine measurement of nuchal translucency in the first trimester of pregnancy will contribute to an increase in the antenatal detection rate of trisomy 21 in the future.

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