European Journal of Medical Genetics 65 (2022) 104404

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg

Management of growth failure and other endocrine aspects in patients with Noonan syndrome across Europe: A sub-analysis of a European clinical practice survey

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ARTICLE INFO

Keywords:
Noonan syndrome
RAS/MAPK signalling Pathway
Clinical practice survey
Europe
Endocrine
Growth hormone

Aim: To date, there is a lack of international guidelines regarding the management of the endocrine features of individuals with Noonan syndrome (NS). The aim was to develop a clinical practice survey to gather information on current treatment and management of these patients across Europe.

Materials and methods: A group of 10 experts from three clinical specialities involved in the management of NS patients (clinical geneticists, paediatric endocrinologists, and paediatric cardiologists) developed a 60-question clinical practice survey. The questionnaire was implemented in Survey Monkey and sent to physicians from these three specialities via European/national societies. Contingency tables and the Chi-Squared test for independence were used to examine differences between specialities and countries.

Results: In total, responses of 364 specialists (paediatric endocrinologists, 40%; geneticists, 30%; paediatric cardiologists, 30%) from 20 European countries were analysed. While endocrinologists mostly referred to national growth charts for the general population, geneticists mostly referred to NS-specific growth charts. Approximately half of the endocrinologists perform growth hormone (GH) stimulation tests in short patients with low IGF1 levels. Two thirds of endocrinologists begin GH treatment for short patients in early childhood (4–6.9 years), and over half of them selected a threshold of −2 standard deviation score (SDS) according to national growth charts. The main concerns about GH treatment appear to be presence of hypertrophic cardiomyopathy (HCM) (59%), increased risk of malignancy (46%), and limited efficacy (31%). When asked if they consider HCM as a contraindication for GH treatment, one third of respondents skipped this question, and among those who replied, two thirds selected 'cannot answer', suggesting a high level of uncertainty. A total of 21 adverse cardiac responses to GH treatment were reported. Although most respondents had not encountered any malignancy during GH treatment, six malignancies were reported. Finally, about half of the endocrinologists expected a typical final height gain of 1–1.5 SDS with GH treatment.

Conclusion: This survey describes for the first time the current clinical practice of endocrine aspects of NS across Europe and helps us to identify gaps in the management but also in the knowledge of this genetic disorder.

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https://doi.org/10.1016/j.ejmg.2021.104404
Received 30 June 2021; Received in revised form 12 November 2021; Accepted 12 November 2021
Available online 9 December 2021

1. Introduction

Noonan syndrome (NS) is a developmental disorder with a roughly estimated incidence of 1 in 2000–2500 live births. This syndrome is caused by germline mutations in genes encoding components or regulators of the RAS/mitogen-activated protein kinase (MAPK) signalling pathway (Roberts et al., 2013; Tajan, M. et al., 2018; Tartaglia et al., 2011). The phenotype varies in severity and can involve multiple organ systems, including distinctive facial appearance, cardiac defects, short stature, skeletal abnormalities, variable cognitive deficits, and predisposition to certain cancers (Tajan, M. et al., 2018).

Short stature affects 50–70% of patients with NS and is one of the main features leading to a diagnosis. Although one quarter of neonates with NS are born small for their gestational age, prenatal growth is usually considered to be only mildly affected with decreased growth velocity and short stature becoming obvious by two years of age (Cessans et al., 2016; Ranke et al., 1988). During childhood, mean height usually follows the lower limit of the normal population, after which it usually declines further because of delayed puberty and a reduced pubertal growth spurt (Ranke et al., 1988). Bone age is usually delayed by about two years throughout childhood, which leads to prolonged catch-up growth at the end of the second decade of life (Ranke et al., 1988; Sharland et al., 1992). In most studies, spontaneous adult height standard deviation score (SDS) is around −2 in females and −2 to −2.5 in males (Cessans et al., 2016; Malaquias et al., 2012; Ranke et al., 1988). However, because of prolonged catch-up growth, reported adult height should be interpreted with caution (Giacomozzi et al., 2015). In recent years, it has been demonstrated that growth patterns in NS significantly vary according to the causative gene (Cessans et al., 2016; Malaquias et al., 2012). Abnormal growth hormone (GH) secretion pattern, partial GH insensitivity, as well as defective chondrocyte differentiation during endochondral bone growth have been suggested to underlie growth retardation, as all of these levels of growth regulation may be affected by aberrant RAS/MAPK signalling (De Rocca Serra-Nedelec et al., 2012; Tajan et al., 2018). Recombinant human GH received approval by the US Food and Drug Administration in 2007 to treat short stature in patients with NS. More recently, this treatment has also been approved by the European Medicines Agency (EMA). With regards to other endocrine features besides delayed puberty, fertility does not seem to be affected in females with NS; however, gonadal dysfunction with deficient spermatogenesis has been described in males (Ankarberg-Lindgren et al., 2011; Moniez et al., 2018). Finally, thyroid dysfunction (Quaio et al., 2012) as well as decrease in bone mass (Baldassarre et al., 2017; Choudhry et al., 2012; Delagrange et al., 2021; Noordam et al., 2002; Stevenson et al., 2011) have also been reported.

To date, there is a lack of international guidelines regarding the screening and the management of the different endocrine aspects of individuals with NS. To describe the current clinical practice across Europe and to identify the gaps and the differences in the management of patients with NS, a clinical practice survey was developed by the European Medical Education Initiative on NS.

2. Methods

2.1. Study design

A group of 10 experts from three clinical specialities closely involved in the management of patients with NS (clinical geneticists, paediatric endocrinologists and paediatric cardiologists) developed a 60-question clinical practice survey on the diagnosis and clinical management of patients with NS. Physicians from these three specialities were invited to participate in this survey, which was implemented using the Survey Monkey platform. Support was provided by several European and National societies for the distribution of the survey. More details regarding the development and distribution of the survey have been published separately (Garcia-Minaur et al., 2021). While the survey contained a number of questions that were applicable to each of the clinical specialties surveyed, there were also speciality-specific subsections designed to collect information on specific aspects of treatment and management. While these sections could be skipped if desired, all survey respondents could potentially view and answer all questions in the survey. Some questions were included within two separate sections of the survey designed to be completed by either paediatric cardiologists or paediatric endocrinologists. As some clinical geneticists answered either one or both of these questions within the cardiology and/or endocrinology subsections, their responses were merged in this analysis. The present article describes the results from questions dealing with the management of endocrine aspects of patients with NS. The responses of paediatric endocrinologists were mainly reported, analysed according to their country of origin, and compared with those of clinical geneticists and paediatric cardiologists where appropriate.

2.2. Statistical analysis

For each question presented in this study, the number of physicians who answered the question was defined along with the percentage of the total number of respondents within that category (*i.e.*, for each speciality). As a second step, only the responses of physicians who gave an answer were considered for the statistical analysis. Differences between specialities and countries were assessed using contingency tables and the Chi-Squared (χ^2) test for independence. The Friedman's test was used for related samples. Given the variable distribution of respondents across countries, responses from endocrinologists and geneticists were only compared between the most represented countries (defined as countries with >10 respondents [paediatric endocrinologists: France, Italy, Germany, Spain, Czech Republic, UK; clinical geneticists: France, Spain, Italy, UK]). All tests were two-tailed and $p < 0.05$ was considered significant throughout the study. Statistical analyses were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA) or IBM SPSS statistic for Windows, version 25.0 (Armonk, NY: IBM Corp, Armonk, NY, USA).

3. Results

3.1. Description of survey respondents

In total, there were 462 respondents from 25 countries. Of these, 98 were removed from this analysis (81 respondents skipped the survey early, 6 were from outside the 28 European countries selected for analysis, and 11 were from other specialities). This resulted in a core analysis set of 364 respondents from 20 European countries (including 18 European Union member states, the United Kingdom and Switzerland). Respondents were evenly distributed across paediatric endocrinology (n = 146, 40%), clinical genetics (n = 108, 30%) and paediatric cardiology (n = 110, 30%). With regards to their country of origin, most respondents were from France (21%), Spain (18%), Germany (16%), Italy (15%), United Kingdom (8%) and the Czech Republic (6%). Similarly to clinical geneticists and paediatric cardiologists, most of the paediatric endocrinologists (69%) were based in university hospitals alone or in combination with other affiliations (general hospital: 19%; private or independent clinic: 7%; specialist centre: 6%). Seventy-four percent of paediatric endocrinologists follow ≤10 patients in an average year, and 92% follow ≤20 patients, which is significantly lower than clinical geneticists (51% follow ≤10 patients and 70% follow ≤20 patients) ($p < 0.0001$).

3.2. Diagnosis of NS

When paediatric endocrinologists were asked about the characteristics that frequently lead to a clinical diagnosis of NS, most indicated that short stature and/or delayed puberty were the leading cause (87% answered with 'frequently [26–50% of cases] or 'most [>50%]').

Concerning the age at referral, most children with NS were referred to endocrinologists during childhood (4–12 years; 63% answered with 'frequently [26–50%] or 'most [>50%]'). Most paediatric endocrinologists (73%) indicated that a clinically evident diagnosis of NS did not mean they were less likely to conduct or order genetic testing (21% said they would less likely perform genetic testing with a clinically evident NS diagnosis, and 6% selected 'cannot answer'). There were significant differences between specialities, with paediatric endocrinologists being less likely to order genetic testing when compared with clinical geneticists ($p = 0.004$).

3.3. Screening and investigation of short stature and indication for GH treatment

Physicians were surveyed on the growth charts that they use for the follow-up of patients with NS. In this analysis, only the responses of clinical geneticists (who refer children with NS and short stature to paediatric endocrinologists) and paediatric endocrinologists (who evaluate children with NS and short stature) were considered. National growth charts and NS-specific growth charts were generally used more often compared with non-specific growth charts (*i.e.*, Centers for Disease Control and Prevention [CDC] and World Health Organization [WHO] growth charts). However, there were differences between both specialities. Paediatric endocrinologists mostly referred to national growth charts for the general population ($p < 0.0001$), whereas geneticists mostly referred to NS-specific growth charts ($p = 0.005$) (Fig. 1A). Although the differences between the top six countries were not significant for paediatric endocrinologists, NS-specific growth charts were more likely to be used by clinical geneticists from the United Kingdom, and less frequently by geneticists from France ($p < 0.0001$).

Regarding hormonal investigation of patients with NS and short stature, the attitude of paediatric endocrinologists was highly variable (and sometimes diverging). Physicians were asked if they usually perform a GH stimulation test in patients with NS and short stature. Among the 63% of paediatric endocrinologists who did not skip the question, half of them (52%) answered that they only performed GH stimulation tests in patients with low insulin-like growth factor 1 (IGF1) levels. Furthermore, 10% answered that they never performed GH stimulation tests, and 36% answered that they always performed a test regardless of IGF1 levels. There were differences between countries with paediatric endocrinologists from Italy more frequently performing GH stimulation test regardless of IGF1 levels ('always' answer) compared with physicians from the Czech Republic and Germany ($p = 0.002$). In contrast, a higher frequency of paediatric endocrinologists from the Czech Republic do not perform any GH stimulation tests ('never' answer) when compared with the other countries ($p = 0.01$) (Fig. 1B).

Interestingly, whatever the differences in the baseline investigation of short stature, more than half of the paediatric endocrinologists (57%) reported that GH deficiency (GHD) is found occasionally (defined as a frequency between 6 and 25% of cases) in patients with NS. There were no significant differences between countries ($p = 0.65$) (Fig. 1C).

Two thirds of paediatric endocrinologists (66%) start GH treatment for patients with NS and short stature in early childhood (4–6.9 years) (Fig. 2A). For one quarter of these physicians (23%), an earlier age would be optimal (1–3.9 years). There were no significant differences across countries ($p = 0.309$).

Regarding the height threshold below which GH treatment should be considered in short children with NS, among the 63% of paediatric endocrinologists who did not skip the question, over half of them (55%) proposed a threshold of −2 SDS and 22% a threshold of −3 SDS according to growth charts for the general population (Fig. 2B). Moreover, 7 out of 16 respondents who answered with 'other criteria' proposed a height threshold of −2.5 SDS. A lower threshold (−3 SDS) tended to be preferred by the paediatric endocrinologists from Germany ($p = 0.057$). Geneticists mostly considered a threshold of −3 SDS according to growth charts for the general population, which was lower compared to paediatric endocrinologists ($p = 0.040$ using the Chi^2 test and $p = 0.051$ using the Fisher's bilateral exact test).

3.4. Concerns about GH treatment

In this survey, it was possible to analyse the main concerns about GH treatment in short children with NS across the three specialities. It is important to note that many responders skipped this question (21% of geneticists, 30% of paediatric endocrinologists, and 24% of paediatric cardiologists), and of those who replied, many could not answer the question (22% of geneticists, 2% of paediatric endocrinologists, and 31% of paediatric cardiologists). Among the 224 remaining respondents (62% of the total number of respondents), the main concerns about GH treatment appear to be the presence of hypertrophic cardiomyopathy (HCM) (59%), the increased risk of malignancy (46%), and the limited efficacy (31%), with important differences across the three specialities (Fig. 3A).

3.4.1. Hypertrophic cardiomyopathy

When physicians were asked about their concerns regarding GH treatment (Fig. 3A), geneticists appeared to be less concerned about HCM when compared with paediatric endocrinologists and cardiologists ($p = 0.041$). Physicians were also asked if they consider HCM a contraindication to the use of GH therapy (Fig. 4). It is important to note that many responders skipped this question (74% of geneticists, 37% of paediatric endocrinologists, and 35% of paediatric cardiologists), and of those who replied, many selected 'cannot answer' (32% of geneticists, 9% of paediatric endocrinologists and 24% of paediatric cardiologists). About one third of respondents from the three specialities (29%) considered that GH treatment is not a contraindication with frequent ultrasound monitoring, and 8% considered HCM not a contraindication at all. Conversely, 19% of respondents considered that GH treatment is a clear contraindication if HCM is present, and 26% considered it a contraindication 'sometimes'. There were no significant differences between the specialities ($p = 0.528$). When asked whether they arrange regular monitoring for patients on GH, most respondents (80%) selected 'yes'.

Physicians were also asked if they have encountered an adverse cardiac response to GH therapy (Fig. 3B). It is important to note that one third of paediatric endocrinologists and paediatric cardiologists skipped this question, and of those who replied, two thirds replied that they could not answer the question. A total of 21 adverse cardiac responses to GH treatment were reported by less than 10% of respondents (10% of paediatric endocrinologists and 7% of paediatric cardiologists).

3.4.2. Risk of malignancies

When physicians were asked about their concerns regarding GH treatment (Fig. 3A), paediatric cardiologists were less concerned about the increased risk of malignancy compared with paediatric endocrinologists ($p < 0.0001$), the geneticists being in an intermediate situation.

Respondents were asked if they had encountered any malignancy in a child with NS receiving GH treatment (Fig. 3C). Among the 92 paediatric endocrinologists who responded to the question, 69 (75%) answered they had not encountered any malignancy, 3 (3%) had encountered a malignancy, and 20 (22%) selected 'cannot answer'. When the 3 paediatric endocrinologists were asked to specify the type of malignancy, two provided an answer (one stated "leukaemia and cerebral dysembryoplastic neuroepithelial tumour [DNET]" and the second

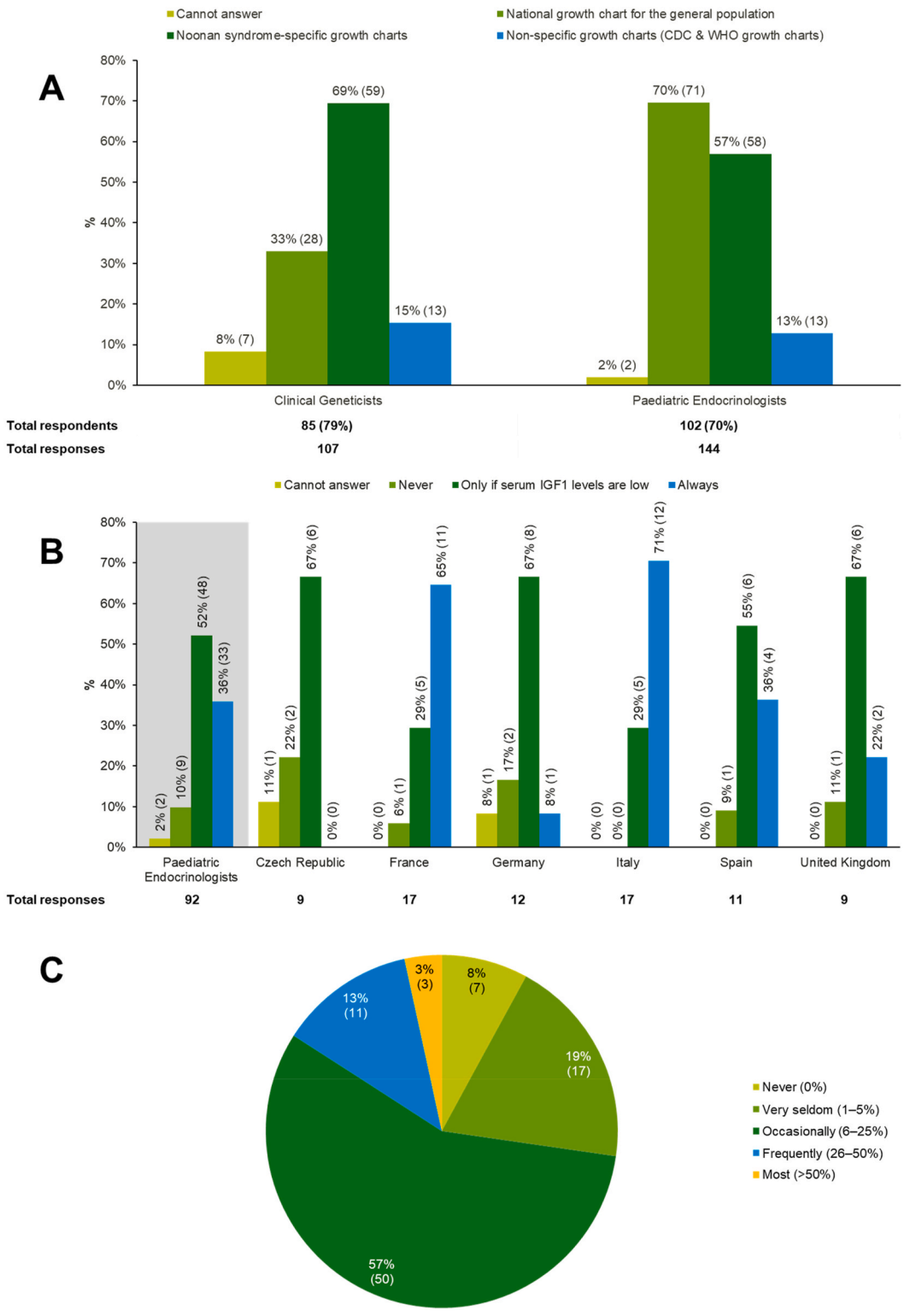

Fig. 1. Screening and investigation of short stature (% [n]).
A. Which growth charts do you use for the follow-up of patients with Noonan syndrome?
Of a total of 254 clinical geneticists and paediatric endocrinologists who responded to the survey, 187 (74%) answered this question. Please note that respondents could provide one or more answers to this question. Percentages shown are calculated from the total number of respondents from each speciality.
B. Do you usually perform a growth hormone stimulation test in patients with Noonan syndrome and short stature?
The results from 92 paediatric endocrinologists (63%) who answered this question are shown on the left of the chart (grey box). Results from 75 paediatric endocrinologists from the top six countries who responded to the survey (62%) are shown on the right. Percentages shown are calculated from the total numbers of respondents from each country.
C. How often do you see growth hormone deficiency in patients with Noonan syndrome?
Shown are the responses from 88 paediatric endocrinologists (60%). Percentages shown are calculated from the total number of paediatric endocrinologists who answered the question.

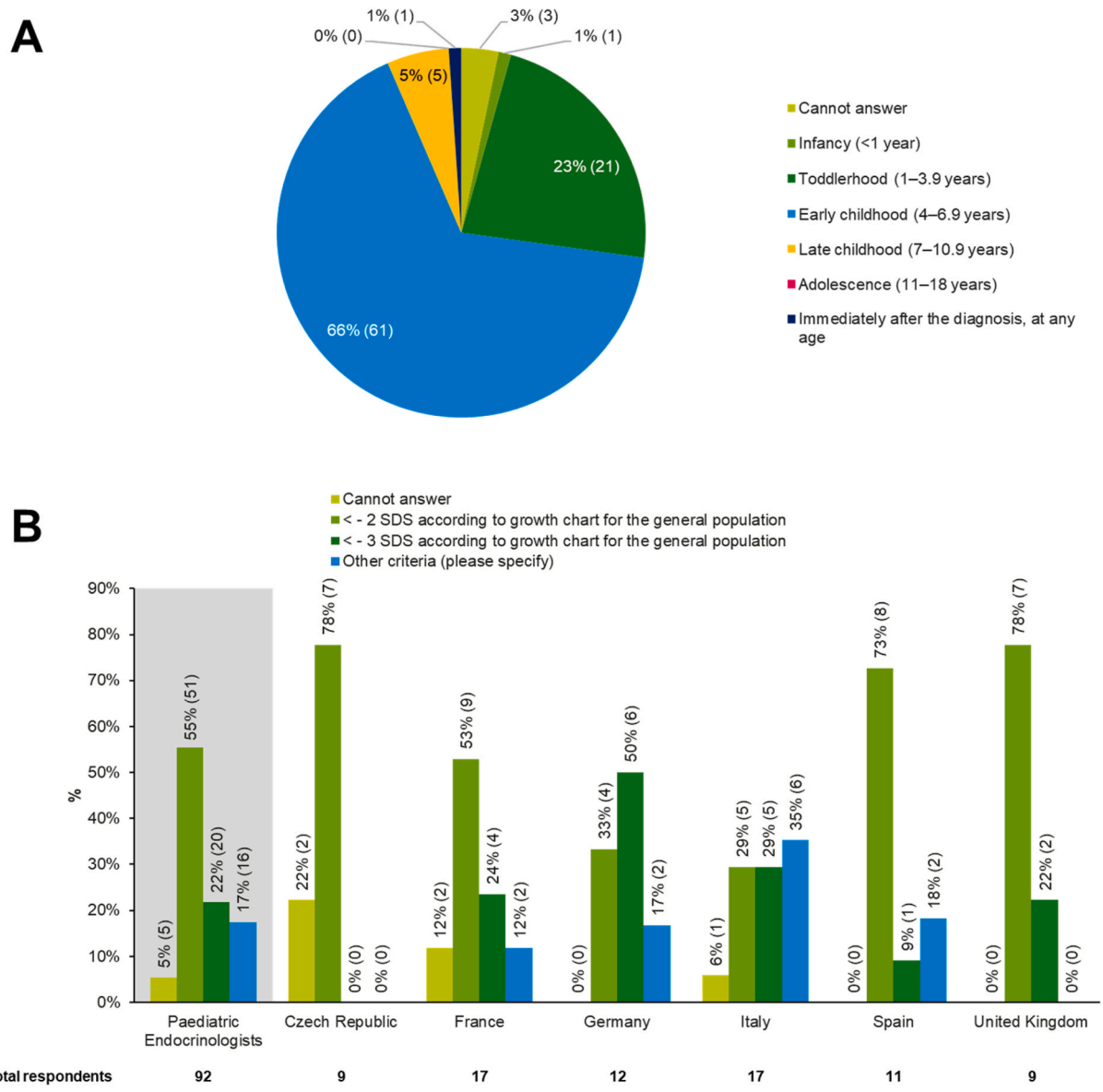

Fig. 2. Indication of GH treatment (% [n]).
A. In your experience, which is the optimal age at which to begin growth hormone treatment for patients with Noonan syndrome and short stature?
Shown are the responses from 92 paediatric endocrinologists (63%). Percentages shown are calculated from the total number of paediatric endocrinologists who answered the question.
B. In your opinion, in the absence of growth hormone deficiency, at what height SDS should you consider growth hormone treatment for patients with Noonan syndrome and short stature?
The results from 92 paediatric endocrinologists (63%) who answered this question are shown on the left of the chart (grey box). Results from 75 paediatric endocrinologists from the top six countries who responded to the survey (62%) are shown on the right. Percentages shown are calculated from the total numbers of respondents, which are depicted below the chart.

stated "DNET cancer"). While this question was contained in the endocrinology subsection of the survey, 16 geneticists (15%) also answered the question. Geneticists also reported three other malignancies, including one juvenile myelomonocytic leukaemia (JMML), one brain tumour and one DNET. Overall, six malignancies were reported by paediatric endocrinologists and geneticists from different institutions: four brain tumours including three DNET, and two cases of leukaemia.

3.4.3. *Limited efficacy*

When physicians were asked about their concerns regarding GH treatment (Figs. 3A), 33% of geneticists and 35% endocrinologists were concerned about limited efficacy. This finding is in accordance with the expected final height gain in patients treated with GH (Fig. 5). Indeed, among the 63% of paediatric endocrinologists who did not skip the question, about half (45%) expect a final height gain between 1 and 1.5 SDS, and one-third (31%) below 1 SDS; this answer was homogenously distributed across countries (no statistically significant differences; $p = 0.439$).

3.4.4. *Other endocrine aspects of NS*

Regarding puberty, which is usually delayed in NS, the need for pharmacological induction was occasional or very seldom as reported by

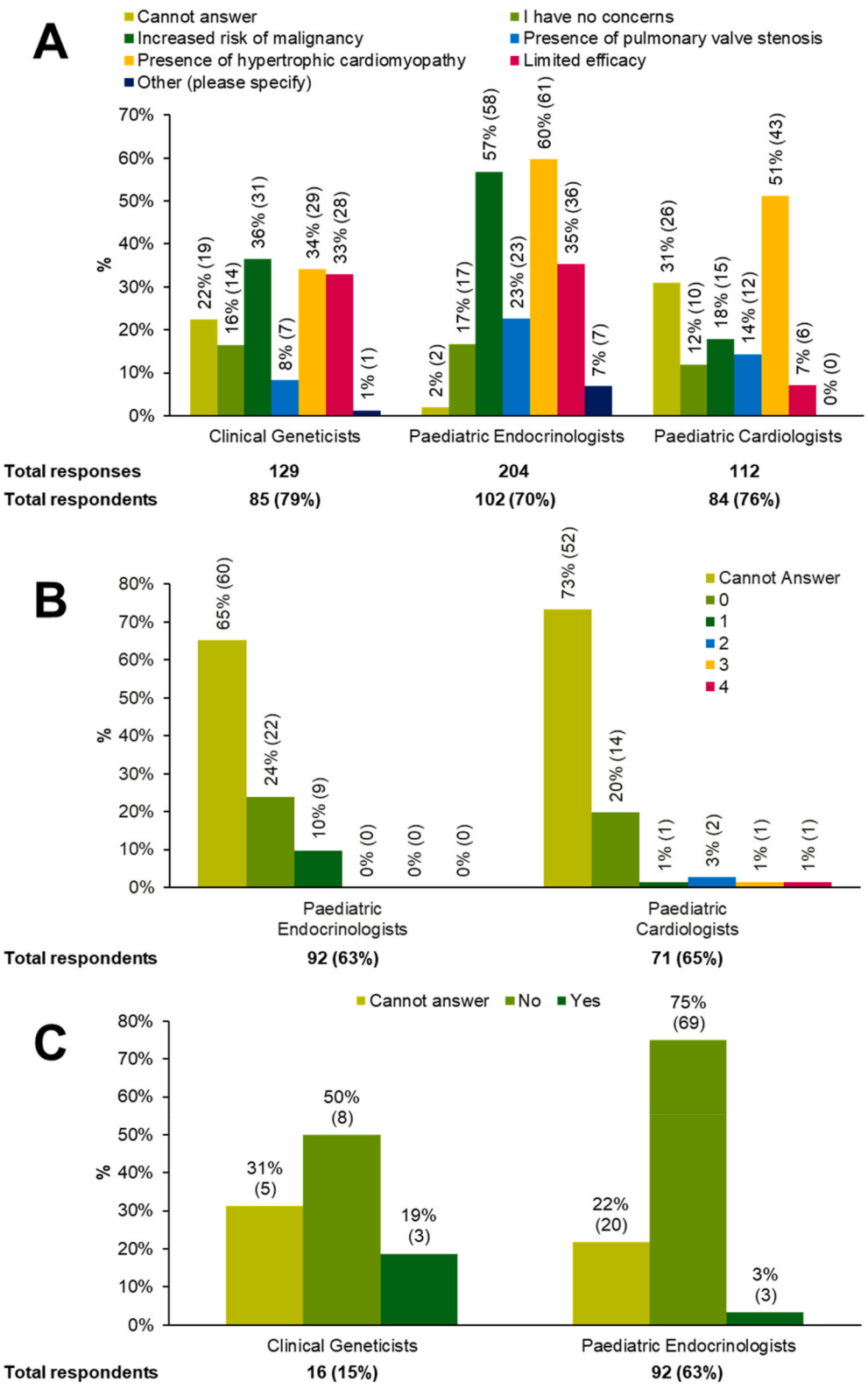

Fig. 3. Concerns about GH treatment (% [n]).
A. Are you concerned about any of the following regarding growth hormone treatment in children with Noonan syndrome?
Of the 364 respondents from the three clinical specialities, 271 (75%) answered this question. Please note that respondents could select one or more options when answering this question. The number of respondents and responses for each specialty is depicted below the chart.
B. How many times have you encountered an adverse cardiac response to growth hormone treatment?
Of the 256 paediatric endocrinologists and paediatric cardiologists responding to the survey, 163 (64%) answered this question. This question was included in two separate subsections of the survey (cardiology and endocrinology). The number of respondents from each specialty is depicted below the chart. One paediatric endocrinologist selected 'other' and responded "Exceptionally" (not included below).
C. Have you encountered any malignancy that developed in a child with Noonan syndrome while they were receiving growth hormone treatment?
Of a total of 254 clinical geneticists and paediatric endocrinologists, 108 (43%) answered this question. Percentages shown in the chart are calculated from the total number of respondents from each speciality.

most physicians (Fig. 6A). There were no differences between patient genders.

Regarding the assessment of fertility in males with NS, it is important to note that 37% of paediatric endocrinologists skipped this question, and of those who replied, many could not answer the question (11%). Most (47%) paediatric endocrinologists perform investigations assessing fertility in males who have experienced cryptorchidism, and one third (28%) in males who have experienced pubertal delay (Fig. 6B). Only 15% of physicians perform investigations assessing fertility in all male patients, regardless of the clinical history.

Thyroid dysfunction was not reported frequently (93% and 99% of respondents answered with 'never and very seldom' or 'occasionally' for

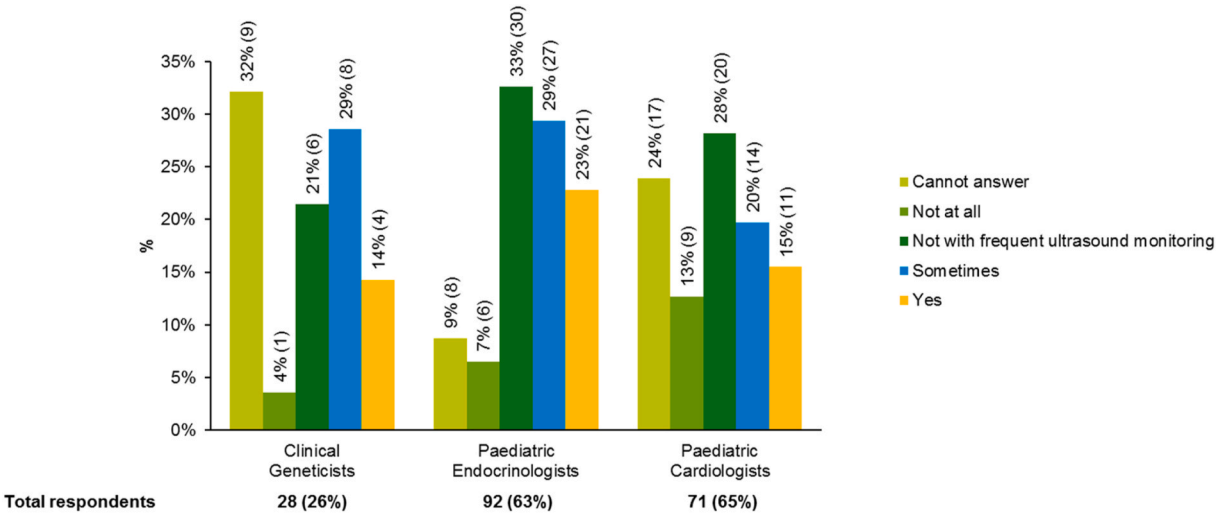

Fig. 4. Do you consider hypertrophic cardiomyopathy a contraindication to the use of growth hormone therapy? (% [n]).
Of the 364 respondents from the three clinical specialities, 191 (52%) answered this question. This question was included in two separate subsections of the survey (cardiology and endocrinology). Responses from clinical geneticists were merged from both questions. The number of respondents from each specialty is depicted below the chart.

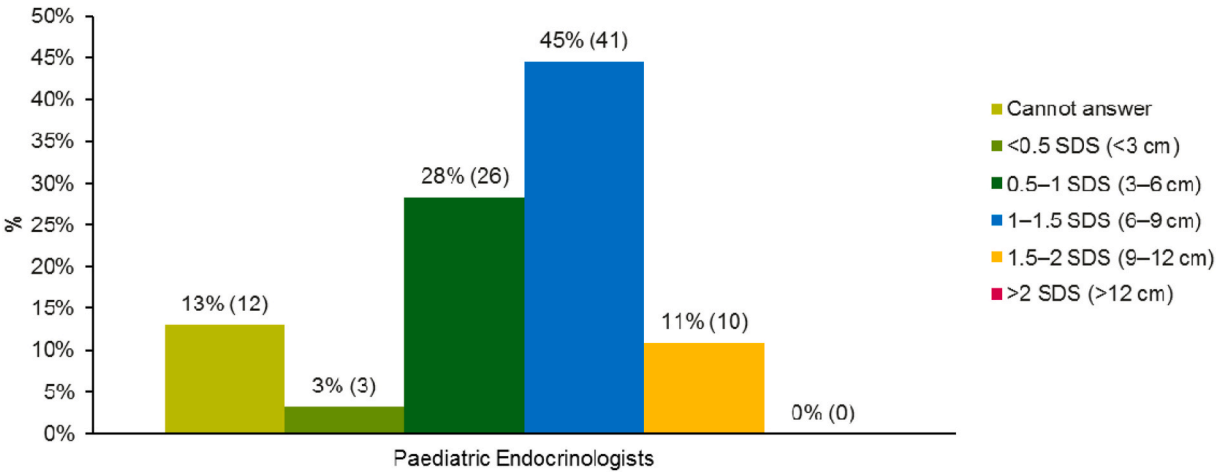

Fig. 5. What is the typical final height gain you would expect in patients with Noonan syndrome treated with growth hormone? (% [n]).

hypothyroidism and hyperthyroidism, respectively) (Fig. 6C).

Finally, one third (29%) of paediatric endocrinologists do not routinely evaluate bone health (*i.e.*, mineral homeostasis and vitamin D, osteodensitometry) in patients with NS, 58% assess mineral homeostasis and vitamin D, and 24% osteodensitometry (Fig. 6D).

4. Discussion

This survey reports a general picture of the current clinical practice for patients with NS across Europe and helps us to identify gaps and differences in their management. The present article describes the results from the survey dealing with the management of endocrine aspects of patients with NS.

4.1. Growth charts

With regards to the growth charts used for the follow-up of patients with NS, there were differences between paediatric endocrinologists, who mostly referred to national growth charts for the general population, and clinical geneticists, who mostly referred to NS-specific growth charts. Although NS-specific growth charts may have significance to describe the natural history of the disease, their usefulness to monitor growth is questionable. Indeed, these NS-specific growth charts were established more than 30 years ago in small cohorts of patients (about one hundred patients) without a molecular diagnosis of NS (Ranke et al., 1988). In recent years, it has been demonstrated that growth patterns in NS are distinct according to the causative gene. Thus, the frequency of short stature (defined as height SDS below −2) varies according to the genotype (*e.g.*, 35% of short stature in *SOS1* patients, 73% in *PTPN11*, 84% in *KRAS*, and 85% in *RAF1*) (supplemental data (Cessans et al., 2016)). As reported by Malaquias et al., patients with mutations in *RAF1* and *SHOC2* genes were shorter than other genotypes, whereas patients with *SOS1* and *BRAF* mutations had more preserved postnatal growth (Malaquias et al., 2012). In the study by Cessans et al., growth retardation was significantly less severe and less frequent at two years of age in patients with *SOS1* mutations compared with patients with *PTPN11* mutations (Cessans et al., 2016). To date, there is no gene-specific growth chart in NS. Finally, the essential issue during follow-up of NS patients is to compare their growth with normally growing children. Consequently, national growth charts for the general population are probably more appropriate to monitor growth in these patients.

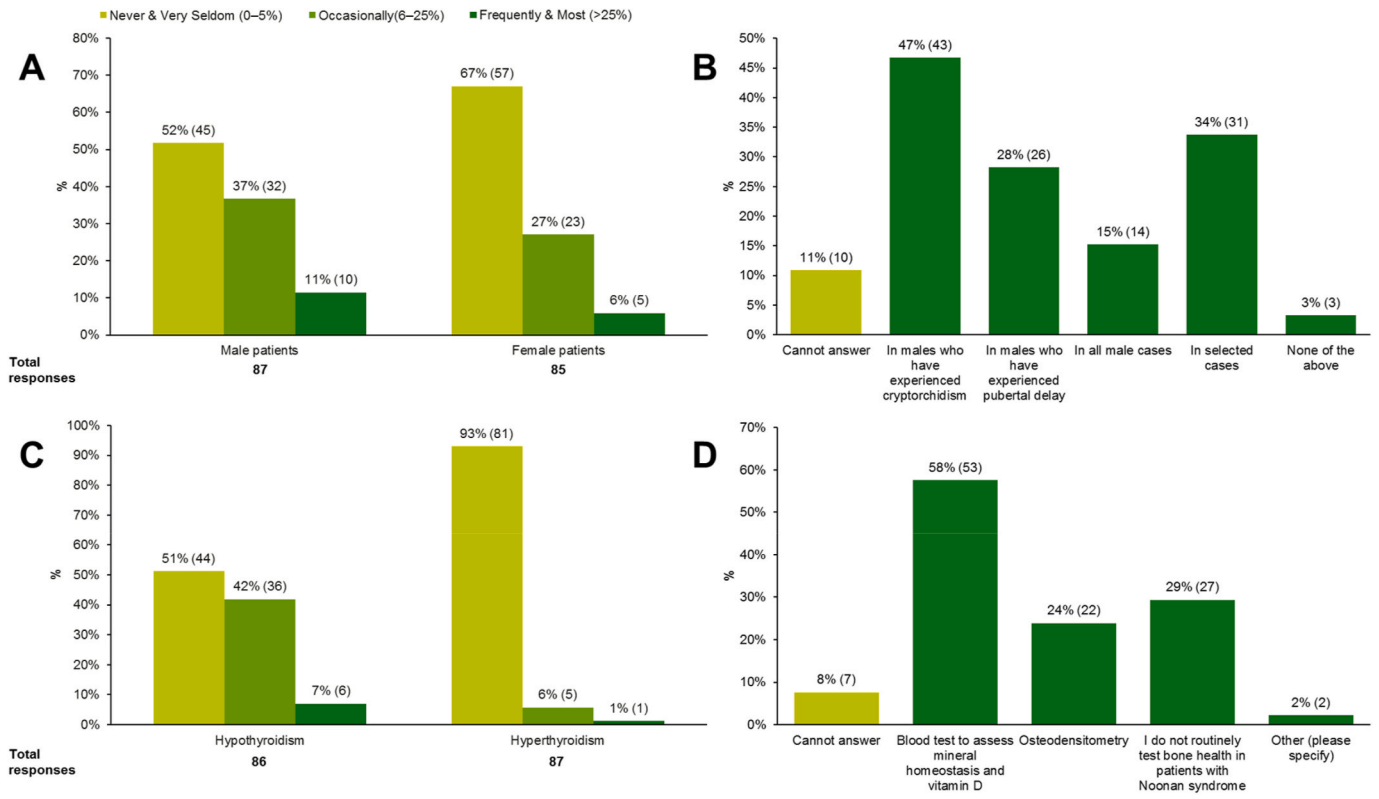

Fig. 6. Other endocrine aspects of NS (% [n]).
Shown are the responses from paediatric endocrinologists (n = 92 [63%]) to the following four questions:
A. Approximately, how frequently have your male/female patients with Noonan syndrome required pharmacological induction of puberty?
B. In which male patients would you routinely perform further investigations assessing fertility?
C. How often do you see thyroid dysfunction in patients with Noonan syndrome?
D. Do you routinely examine bone health in patients with Noonan syndrome? If so, which investigations do you perform?
In panels A and C, percentages are calculated from the total number of responses in each category (respondents could answer in one or both categories).
In panels B and D, one or more answers could be selected.

4.2. Hormonal investigation

Regarding investigations performed in NS patients with short stature, the attitude of paediatric endocrinologists was highly variable (and sometimes diverging) across countries. Indeed, although half of the physicians performed GH stimulation tests in patients with low IGF1 levels, some of them never performed these tests, whereas others always performed these tests regardless of IGF1 levels. In view of the mechanisms underlying short stature in patients with NS, the usefulness of GH stimulation tests is disputable. Indeed, while there have been reports on GHD (Cotterill et al., 1996; Romano et al., 1996) or neurosecretory dysfunction (Ahmed et al., 1991; Noordam et al., 2001b; Tanaka et al., 1992), patients with NS (notably those with *PTPN11* mutations) usually display normal or slightly increased GH levels associated with low serum IGF1 levels, suggesting GH insensitivity (Binder et al., 2005; Limal et al., 2006). GH insensitivity, as well as abnormal chondrocyte differentiation, both involving hyperactivation of the RAS/MAPK signalling pathway, were confirmed *in vivo* in transgenic mice expressing a NS-associated *Ptpn11* allele (De Rocca Serra-Nedelec et al., 2012; Tajan et al., 2018). Interestingly, whatever the differences in the baseline investigation of short stature reported in this survey, more than half of the paediatric endocrinologists reported that GHD is found occasionally in patients with NS. In fact, before GH was approved for NS *per se*, this treatment could only be used for NS patients with growth retardation associated with GHD, or for children born small for gestational age. As a result, GH testing has probably been used more frequently in order to find an indication for GH treatment. With the GH now being approved for NS in Europe, GH testing is expected to decrease significantly.

4.3. Height threshold and optimal age to start GH treatment

There were differences in the height threshold below which GH treatment should be considered in NS children with short stature, with clinical geneticists considering a lower threshold compared with paediatric endocrinologists. The prolonged catch-up growth at the end of the second decade that has been well documented in NS (Ranke et al., 1988; Sharland et al., 1992) may be a reason for some specialists to apply a lower threshold at the start of treatment. This prolonged growth and late catch-up growth should lead to cautious interpretation of reported GH treatment efficacy on adult height, because in non-controlled studies, this effect leads to an overestimation of the GH effect size (Giacomozzi et al., 2015).

Two thirds of paediatric endocrinologists proposed to start GH treatment for NS patients with short stature in early childhood (4–6.9 years). For one quarter of these physicians, the optimal age would be earlier (1–3.9 years); it is likely that this answer includes the age group 3–4 years, which was not individually specified in the survey. The determination of optimal age to start GH treatment is a challenging issue and should take into account the nutritional status and potentially the motor development of these children. Indeed, feeding difficulties (such as a weak sucking, prolonged feeding time or recurrent vomiting) are reported in three quarters of infants with NS and can contribute to failure to thrive (Sharland et al., 1992; Shaw et al., 2007). Although these feeding problems usually resolve in the first months/years of life, about one quarter of these infants require tube feeding for two weeks or longer. In NS patients with the most severe failure to thrive in infancy, there is probably a nutritional contribution to short stature. In other

syndromes associated with failure to thrive such as Silver-Russel syndrome, international guidelines have stressed that the main therapeutic goals for the first two years of life should be nutritional support and recovery of any calorie-related length or height deficit, which should be addressed before initiation of GH therapy (Wakeling et al., 2017). On the other hand, early GH administration may have a potential beneficial effect on muscular hypotonia and motor development as it has been reported in patients with Prader-Willi syndrome (Reus et al., 2014). Further prospective trials in infants with NS are needed to address and clarify this issue.

4.4. Concerns about GH treatment

The main concerns about GH treatment appear to be the presence of HCM, the increased risk of malignancy, and limited efficacy, with important differences according to the three specialities. A significant proportion (12–17%) of respondents had no concerns.

4.4.1. Hypertrophic cardiomyopathy

Patients with NS are predisposed to cardiac diseases, notably pulmonary valve stenosis and HCM, occurring in 50–60% and 20% of patients with NS, respectively, with variable prevalence depending on the gene involved and the specific type of mutation (Calcagni et al., 2017; Prendiville et al., 2014). Given the known stimulating effect of the GH/IGF1 axis on cardiac mass (Twickler et al., 2004), concerns have been raised about a possible increased risk of alteration in cardiac function in patients with NS treated with GH, particularly the development or deterioration of HCM. In this survey, there are significant discrepancies between respondents regarding the meaning of HCM as a contraindication for GH. These discrepancies may be attributed in part to the lack of a clear definition of the term HCM in the survey, which may imply a very broad clinical spectrum ranging from mild myocardial hypertrophy without any functional impairment to severe progressive HCM. It is also important to note that one third of respondents skipped this question, and of those who replied, two thirds replied that they could not answer the question, which suggests a high level of uncertainty; it is notable, therefore, that adverse cardiac events were reported in this survey from a small number of respondents. This uncertainty is likely due to a lack of robust data in the published literature regarding this particular question. Two short-term studies specifically addressing the effect of GH on cardiac function in NS did not find any effects (Cotterill et al., 1996; Noordam et al., 2001a). Similarly, two long-term prospective trials of GH therapy in NS did not find any cause for concern, but children with known HCM were excluded from these trials (Noordam et al., 2008; Osio et al., 2005). In contrast, Horikawa et al. included altogether eight patients with HCM in a long-term study of two different dose-regimes and did not report any ultrasound data on the effect of GH in these patients. In this study, altogether nine adverse cardiac responses were reported, four of those arrhythmias (three in high-dose group and one in low-dose group), all in patients with pre-existing but not specified cardiac disease (Horikawa et al., 2020). Several post-marketing observational studies including a large number of patients with NS treated with GH provide interesting data because a significant proportion of these patients had cardiac defects at baseline; however, the precise description of the type and the severity of cardiac defect is often lacking in these studies. Of the 429 patients with NS included in the Kabi International Growth Study (KIGS) database, seven cardiac adverse events were reported during the course of GH therapy, including cyanotic periods (one patient), cardiac arrhythmias (three patients), angina pectoris (one patient), left ventricular hypertrophy (one patient), and cardiomyopathy requiring heart transplantation (one patient) (Otten and Noordam, 2007); the pre-existing cardiac situation was not described in this article. Of the 370 patients with NS included in the National Cooperative Growth Study, 46% had cardiac disorders (Romano et al., 2009). During the course of GH therapy, three cardiac adverse events were reported (increased biventricular hypertrophy, HCM, and supravalvular aortic stenosis), but all of them were considered to be comorbidities of NS. Finally, of the 412 patients included in two complementary non-interventional studies (NordiNet IOS and ANSWER), 41 patients had cardiovascular comorbidities, including congenital pulmonary stenosis (19 patients), HCM (3 patients), and unspecified congenital malformation of the heart (3 patients) (Rohrer et al., 2020). No other cases of HCM or other cardiopathies were reported during GH therapy.

These safety data are reassuring in patients with NS without known HCM, as long as cardiac status is monitored while on GH therapy. However, current knowledge is insufficient to conclude about the safety of GH treatment in patients with NS and pre-existing HCM (even mild).

4.4.2. Increased risk of malignancy

In this survey, most respondents answered they had not encountered any malignancy during GH treatment. However, it is important to note that many respondents skipped this question, and of those who replied, most replied that they could not answer the question. Overall, six malignancies were reported in this survey: four brain tumours including three DNET, and two leukaemias. Patients with NS are predisposed to have a higher risk than the general population for leukaemia (*e.g.*, juvenile myelomonocytic leukaemia) and solid tumours, including neuroblastoma and central nervous system tumours such as DNET. The cumulative risk of developing cancer was 3.5-fold higher among individuals with a *PTNPN11* mutation (Jongmans et al., 2011) and 8.1-fold higher among individuals with molecularly confirmed NS than in the general population (Kratz et al., 2015). This raises concerns about the use of GH treatment in patients with NS. A literature review performed by McWilliams et al. identified nine reports of DNET and 13 non-DNET brain tumours in individuals with NS (McWilliams et al., 2016) in this series, tumour growth while receiving GH occurred in two patients. Bangalore Krishna et al. described two other NS patients who developed brain tumours while being on GH treatment (Bangalore Krishna et al., 2017) Molecular diagnosis of NS is usually lacking in previous case reports and it may be hypothesized that specific mutations may convey higher tumour risks. Jacquinet et al. reported a 22-year-old patient with NS related to a *LZTR1* mutation who developed a ganglioblastoma; this patient had been transiently treated with GH between ages 15 and 17 (Jacquinet et al., 2020). Finally, in the post marketing observational studies published by Rohrer et al., four events were reported in three patients (3/412; 0.7%) including brain neoplasm (reported as a serious adverse event [SAE] unlikely to be related to GH treatment) in one patient, glioneuronal tumour (reported as an SAE unlikely to be related to GH treatment) in one patient, and one event each of brain neoplasm and metastases to the spine both reported as a serious adverse reaction (possibly related to GH treatment) in one patient (Rohrer et al., 2020). Given the estimated risk of 1–2% for paediatric malignancies in patients with NS (Kratz et al., 2015), it is currently unclear whether reported cases of malignancy occurring during GH treatment do significantly exceed this baseline risk. Long-term studies taking into account patient stratification based on the mutated gene and type of mutation are needed to assess differences in incidence and the relationship with GH therapy.

4.4.3. Limited efficacy

One third of physicians were concerned about the limited efficacy of GH treatment. This finding is in accordance with the expected final height gain in patients treated with GH. Indeed, about half of paediatric endocrinologists expect a final height gain between 1 and 1.5 SDS, and one-third below 1 SDS in patients with NS treated with GH. Although short-term trials on GH treatment in children with NS and short stature have reported an enhancement of growth velocity and mean height standard deviation (Ahmed et al., 1991; Cotterill et al., 1996; De Schepper et al., 1997; Lee et al., 2012; MacFarlane et al., 2001), the benefit on adult height is still uncertain. To date, nine studies have reported data on adult height or near adult height (NAH) outcomes in

patients with NS treated with GH (Kirk et al., 2001; Malaquias et al., 2019; Noordam et al., 2008; Osio et al., 2005; Raaijmakers et al., 2008; Ranke et al., 2019; Rohrer et al., 2020; Romano et al., 2009; Tamburrino et al., 2015). However, these trials are difficult to compare because of differing protocols (variations in age at start of treatment, doses and duration of treatment) and outcome criteria (different definitions of NAH). None of these studies were randomised controlled trials and all were significantly biased (Giacomozzi et al., 2015). In these studies, the height gain SDS on final height ranged from 0.6 to 1.7 (about 5–10 cm respectively), which is comparable to those reported in other non-GHD indications of GH treatment such as Turner syndrome and SHOX deficiency. All these studies reported a high individual variability of response to GH treatment. Similarly to genotype-phenotype correlations reported for spontaneous growth, different genotypes may explain part of this variability. However, in most of the studies, the diagnosis of NS was made clinically without molecular confirmation, which does not allow for investigation of genotype-phenotype correlations. Although a lower growth response was initially suggested in patients with a *PTPN11* mutation (Limal et al., 2006), this result was not confirmed in long-term studies with a similar height gain compared to other genotypes (Noordam et al., 2008). In a study by Tamburrino et al., patients harbouring a *SHOC2* mutation showed a better response to GH treatment than patients with *PTPN11* mutations (Tamburrino et al., 2015). Earlier initiation of GH treatment and longer pre-pubertal duration of therapy were associated with improved efficacy of GH therapy (Osio et al., 2005; Ranke et al., 2019; Romano et al., 2009). Finally, a recent 4-year randomized, double-blind, multi-centre trial investigating the effect of two doses of GH (66 versus 33 μg/kg/day) in pre-pubertal children with NS demonstrated a significant improvement in height gain with the higher dose (Horikawa et al., 2020).

Long-term data on the effect of GH treatment on height outcomes and on the factors modulating this effect are needed in a large cohort of patients with NS. Notably, further prospective studies are needed to investigate possible genotype-phenotype correlations in terms of efficacy and safety (including HCM and risk of malignancy) of GH therapy. In order to answer this key question, a genetic diagnosis should be available in all NS patients. As a result, paediatric endocrinologists must be aware of the importance of genetic testing. Regarding this issue, it should be stressed than 15% of physicians (21% of paediatric endocrinologists) responding to this survey were less likely to perform genetic testing in patients with a clinically evident diagnosis of NS. One explanation for this could be that access to genetic testing services and associated costs could be problematic for physicians in some countries. However, results from the survey published in the companion manuscript by Garcìa-Minaùr et al. (Garcia-Minaur et al., 2021) indicated that reimbursement was not a limiting factor for geneticists completing the survey. There is a need for the creation of coordinated European registries to collect data on the evolution of these patients, the medical complications that may arise during their lives and the response and possible side effects of growth hormone treatment.

Although classically mentioned in NS, some features, such as the need of pharmacological induction for delayed puberty and the higher risk of thyroid dysfunction, were occasionally reported by the paediatric endocrinologists, which is in accordance with the published data. Thus, in a large cohort of patients with NS reported by the Shaw et al. study, delayed puberty was induced by sex steroids hormones in only 6% of patients (Shaw et al., 2007). Similarly, in a cohort of 42 patients with NS and other RASopathies, Quaio et al. reported a frequency of 7% of overt hypothyroidism requiring hormone replacement, which was only slightly higher than those observed in the general population (Quaio et al., 2012).

Regarding the assessment of fertility and bone health in patients with NS, only half of the physicians were able to answer this question, suggesting a high level of uncertainty. Sertoli cell-specific primary testicular insufficiency and infertility have been reported in patients with NS (Ankarberg-Lindgren et al., 2011; Moniez et al., 2018); However, the exact frequency of infertility in adult males is unknown. Similarly, although several studies have reported decreased bone mass in patients with NS patients and other RASopathies (Baldassarre et al., 2017; Choudhry et al., 2012; Delagrange et al., 2021; Noordam et al., 2002; Stevenson et al., 2011), the long-term risk of bone fragility in adults and the elderly is unknown. More generally, although the features of NS are well described during childhood, little is known about the progression of the disease in adulthood. Prospective long-term follow-up studies are required to further investigate fertility, bone health and also metabolic status in NS adults and to clarify the long-term follow-up of these patients.

5. Conclusion

This survey describes the current clinical practice of endocrine aspects of NS across Europe and helps us to identify the gaps in the management and also in the knowledge of this genetic disorder. This underlines the importance to establish international guidelines regarding the screening and the management of the different endocrine aspects of individuals with NS. Moreover, further data are needed to investigate some unresolved issues, such as possible genotype-phenotype correlations in terms of efficacy and safety of GH treatment in patients with NS (including height outcomes, HCM, and risk of malignancy).

Financial support and sponsorship

The initiative was supported by an unrestricted grant from Novo Nordisk Europe A/S, with Steering Committee members receiving honoraria for survey development. Novo Nordisk has had no influence on the scientific content or materials generated as part of this project. MZ, MT and TE are recipients of EJP-RD funding (NSEuroNet) (grant number for MZ: FKZ 01GM1921A). MZ also received funding from the German Federal Ministry of Education and Research (BMBF) for the project GeNeRARe (German Network for RASopathy Research; FKZ: 01GM1902A). JL is a recipient of AZV grant No. NV18-07-00283.

Declaration of interest

T. Edouard: Research Support: Novo Nordisk, Pfizer, Sandoz (speakers honoraria paid to INSERM unit for research use). M. Zenker: Speakers Bureau/Honoraria: Novo Nordisk, AstraZeneca; Consultant/Advisory Board: Novo Nordisk, FDNA. I. Östman-Smith: Research Grants: Swedish Heart-and Lung Foundation, and the Swedish state under the agreement between the Swedish government and the County councils, the ALF-agreement. E. Ortega Castelló: None. C.M. Wolf: Research Grants: Deutsche Herzstiftung; Speakers Bureau/Honoraria: Novo Nordisk; Consultant/Advisory Board: Novo Nordisk. E. Burkitt-Wright: Speakers Bureau/Honoraria: Novo Nordisk, Springer. A. Verloes: None. S. García-Miñaúr: None. M. Tartaglia: Speakers Bureau/Honoraria: Novo Nordisk; Consultant/Advisory Board: Novo Nordisk. G. Shaikh: Research Grants: Soleno Therapeutics, Inc, OPKO Health; Speakers Bureau/Honoraria: Novo Nordisk, Pfizer, Ferring. J. Lebl: Speakers Bureau/Honoraria: Novo Nordisk, Pfizer, Merck, Sandoz.

Author contributions

TE, MZ, IÖS, CMW, EBW, AV, SGM, MT, GS and JL contributed to development of the survey questions, analysis of the results, and drafting of the manuscript. EOC performed statistical analysis. All authors have read and approved the final version of the manuscript for submission.

Data availability statement

All of the data supporting the results presented in this paper are available on request.

The results from 92 paediatric endocrinologists (63%) who answered this question are shown. Percentages shown are calculated from the total number of respondents (paediatric endocrinologists).

Acknowledgments

We would like to acknowledge the following societies for their valuable assistance with distribution of the survey: British Society for Genetic Medicine, British Society for Paediatric Endocrinology and Diabetes, British Congenital Cardiac Association, French Paediatric Endocrinology and Diabetology Society (SFEDP - Société Française d'Endocrinologie et Diabétologie Pédiatrique), French Cardiology Society (Société Française de Cardiologie), French Society of Clinical Genetics (FFGH - Société Française de Génétique), ERN ITHACA Network, Belgian Paediatric Endocrinology Society, German Society for Human Genetics (GfH), German Society for Paediatric Endocrinology (DGKED), German Society of Paediatric Cardiology and Congenital Heart Disease (DGPK), Austrian Society for Child and Adolescent Medicine (ÖGKJ), Working Group Paediatric Cardiology, Paediatric Endocrinology, and Paediatric Human Genetics, Swiss Association of Paediatric Cardiologists, Swiss Paediatric Endocrinology Society, Spanish Association of Human Genetics (AEGH - Asociación Española de Genética Humana), Spanish Paediatric Cardiology Society (SECPCC - Sociedad Española de Cardiología Pediátrica y Cardiopatías Congénitas), Spanish Paediatric Endocrinology Society (SEEP - Sociedad Española de Endocrinología Pediátrica), Swedish Society of Paediatric Cardiologists, Italian Society Paediatric Genetic Diseases and Congenital Disabilities (SIMGePeD Società Italiana Malattie Genetiche Pediatriche e Disabilità Congenite), Italian Society of Paediatric Endocrinology (SIEDP - Società Italiana di Endocrinologia e Diabetologia Pediatrica), Italian Society of Human Genetics (SIGU - Società Italiana di Genetica Umana), Italian Paediatric Society (SIP - Società Italiana di Pediatria), Czech Paediatric Society (ČPS), Section of Paediatric Endocrinology, Association for European Paediatric and Congenital Cardiology. The authors are extremely grateful to the physicians who completed the survey. Several authors are member(s) of the European Reference Network on Rare Congenital Malformations and Rare Intellectual Disability ERN-ITHACA (EU Framework Partnership Agreement ID: 3HP-HP-FPA ERN-01-2016/739516) and of the European Reference Network on rare endocrine conditions (Endo-ERN). We would like to thank Novo Nordisk Europe A/S for funding. We are also grateful to Nick Fulcher, Chrissie Kouremenou and Monika Schrimpf, from Ashfield MedComms GmbH (Mannheim, Germany) for their assistance with survey development and implementation, analysis of survey results, and manuscript development. We wish to acknowledge in particular Nick Fulcher for his valuable help in the design of the survey, the formatting of the figures and language editing.

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