Short and long-term outcomes of children with autoimmune congenital heart block treated with a combined maternal-neonatal therapy. A Comparison study

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Running head: A combined therapy for congenital heart block

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ABSTRACT

Objective The short and long-term outcomes of children with anti-Ro/La-related congenital heart block treated with a combined maternal-neonatal therapy protocol were compared with those of controls treated with other therapies.

Study design Sixteen mothers were treated during pregnancy with a therapy consisting of daily oral fluorinated steroids, weekly plasma exchange and fortnightly intravenous immunoglobulins and their neonates with intravenous immunoglobulins (study group); 19 mothers were treated with fluorinated steroids alone or associated to intravenous immunoglobulins or plasma exchange (control group).

Result The combined-therapy children showed a significantly lower progression rate from 2^{nd} to 3^{rd} degree block at birth, a significant increase in heart rate at birth and a significantly lower number of pacemaker implants during post-natal follow-up with respect to those treated with the other therapies.

Conclusion The combined therapy produced better short and long term outcomes with respect to the other therapies studied.

INTRODUCTION

Autoimmune congenital heart block (CHB), a rare disease occurring in 1 out of 23 000 births in the general population [1], is an autoantibody-mediated disorder potentially caused by placental transmission of maternal autoantibodies to SSA/Ro and/or SSB/La ribonucleoproteins, which can induce inflammation and subsequent fibrosis in the atrioventricular (AV) node and myocardial tissue [2]. Some cardiac complications such as myocarditis, pericardial effusions, fetal hydrops, endocardial fibroelastosis and dilated cardiomyopathy can complicate CHB [3, 4]. It has been found that the risk for CHB, which is 1-5% in anti-Ro/La antibody-positive pregnancies, rises to 12-20 % for women with a previously affected child [5]. CHB is generally diagnosed in patients with Sjögren's syndrome, systemic lupus erythematosus or undifferentiated connective tissue diseases, but it has also been detected in asymptomatic pregnant women [5].

The first signs of CHB such as persistent fetal bradyarrhythmia are usually noted between the 18^{th}–24^{th} weeks of gestation [2]. Despite frequent reports of cardiac conduction returning to a normal both in treated and untreated fetuses in whom signs of 1^{st} degree heart block have been detected [6], CHB appears to be a rapidly progressive disease, and it is unclear if regression from a 2^{nd} degree AV block is possible [7, 8]. Regardless of the type or amount of treatment utilized, no recovery from a 3^{rd} degree or complete heart block has ever been reported [7, 8]. This condition is potentially a lethal one associated with a high rate (20%) of fetal/neonatal mortality generally due to severe cardiomiopathy/endocardial fibroelastosis and a high rate of infant morbidity with more than 80% of survivors requiring a pacemaker, and, in rare cases, heart transplantation [4, 5, 9, 10].

Although there are no standard guidelines for the management of CHB, treatment mainly consists in fluorinated steroids (dexamethasone or betamethasone) [11]. While treating early signs of 2^{nd} degree CHB with fluorinated steroids together with intravenous immunoglobulins (IVIG) seems to induce regression of an incomplete CHB [12], there is no evidence that the use of antenatal fluorinated corticosteroids alone can improve fetal/neonatal CHB morbidity or mortality [13, 14]. A recent clinical trial reported that the use of hydroxychloroquine was efficacious, but for preventing CHB recurrence in anti-SSA/Ro-positive mothers with a previous pregnancy complicated by CHB [15].

A therapeutic protocol consisting in (i) plasma exchange + IVIG infusions + oral betamethasone,

administered to women from the time fetal CHB was detected and throughout the rest of the pregnancy, and (ii) IVIG, administered to the neonates after birth, was first described by our group in 2012 [16]. The results obtained over this past decade have been encouraging and have led us and other specialized medical centers to use it when CHB was diagnosed [17-22]. The current study reports on the short and long-term outcomes associated to the protocol, compares them with those associated to therapies based on fluorinated steroids alone or together with IVIG or plasma exchange, and evaluates its advantages, limits, and safety profile.

SUBJECTS AND METHODS

Study population

The study's inclusion criteria were the following: (i) maternal positivity to anti-SSA/Ro ± anti-SSB/La antibodies; (ii) intrauterine echocardiographic detection of 2^{nd} or 3^{rd} degree fetal CHB not associated to structural congenital heart disease; (iii) receiving therapy starting from the time CHB was detected until delivery. The patients' clinical diagnoses were formulated by rheumatologists staffing the outpatient clinic of the Rheumatology Unit of the University of Padua Medical Center. Two therapy protocols were used: the women in the study group were treated with a maternal-neonatal combination therapy; the women in the control group were treated with fluorinated steroids alone or associated to IVIG or plasma exchange. All of the women underwent a physical examination, fetal ultrasound studies, and routine biochemistry testing every two weeks from the time therapy was begun until delivery. Fetal echocardiographies were weekly performed by a single pediatric cardiologist who was a member of the staff of the Pediatric Cardiology Unit of the University of Padua Medical Center, starting from the time CHB was detected to the end of the pregnancy. The indication criteria for pacing chidren with autoimmune complete CHB was made in accordance with literature data available at the time of the study [23-25] and were the following: There is no argument that any symptomatic bradycardia patients (i.e. with syncopal episodes or limited exercise capability or congestive heart failure) require pacing. It is generally accepted that a mean awake resting heart rate below a determined number for the age group is an indication for pacing. This is frequently quoted as a 55 beats/minute in the newborn period and gradually decreases with advancing age (50 beats/minute in toddlers and children). Other strong indications are considered: significant pauses on 24-hour electrocardiographic monitoring (>3 seconds while awake or >5 seconds while sleeping), a prolonged QTc

(longer than 460 msec), a wide QRS escape rhythm or complex ventricular ectopy (couplets or greater). Echocardiograms may be helpful also to determine progressive loss of systolic function of the ventricle with increasing heart size and the development of mitral regurgitation, any of which would be considered a relative indication for pacing. The patient who did not receive a pacemaker have to be reevaluated at least yearly with an electrocardiogram, Holter monitoring, echocardiography and possibly exercise stress testing. At the end of the follow-up, the mothers belonging to the study group were asked to fill out a dedicated questionnaire concerning the general health and quality of life of their children. Trend over time of CHB degree, of heart rate and of cardiomyopathy/endocardial fibroelastosis lesions and the numbers of pacemaker implants and of deaths were the study's primary outcomes. Pregnancy complications, gestational age at delivery, birth weight in percentiles and infants'Apgar score at 5 minutes were secondary outcomes. The primary and secondary outcomes of the study group were compared with those of the control group. The study was approved by the institutional review board for observational studies and the Audit Committee of the University-Hospital of Padua (# 6894) and was carried out in accordance with the 1964 Declaration of Helsinki and its later amendments, or comparable ethical standards. Once the patients were informed about the disease risks and the potential risks/benefits of the treatments, they were asked to sign informed consent forms.

The therapy for the study group

The treatment protocol used for the study group referred to maternal and as well as neonatal therapies.

Maternal treatment. Oral betamethasone (8 mg/day) for three consecutive days was prescribed to the women at the time the fetuses were diagnosed with CHB, then 4 mg/day continuously until delivery. Following birth betamethasone was switched to prednisone (25 mg/day) and was gradually tapered over the puerperium period unless the mother's clinical condition required continuous steroid treatment. A high rate of oligohydramnios and intrauterine growth restriction (IUGR) (7, 58.3%) probably due to steroid treatment were registered in the first 12 treated patients. Given these complications and relying on the efficacy of the other associated therapies, starting in August 2015 betamethasone was reduced to 8 mg/day for three consecutive days, then 4 mg/day until the 28^{th} week of gestation, then 2 mg/day until delivery. This protocol was utilized for the last four patients included in this study (cases n. 13-16). In order to evaluate the

uniformity of the study group treated with combined therapy the clinical characteristics, antibody profiles and outcomes of the 12 mothers treated with the previous combined therapy were compared with those of the four mothers treated with the modified combined therapy. Plasma exchange sessions were performed using a COBE Spectra (Terumo BCT, Lakewood, Co, USA) continuous blood cell flow separator according to the following timetable: at onset sessions were daily for the first two days and weekly thereafter until the time of delivery. As has been demonstrated, plasma exchange performed once a week led to a significant decrease in maternal pathogenic antibody levels [26]. The last session was performed the day before the planned delivery date. Seventy to 100% of the plasma volume was exchanged at each session; the replacement fluid was a mixture of 70% albumin (4%) and 30% saline. Acid Citrate Dextrose Formula A anticoagulant used in a 1:12-1:15 ratio was utilized to ensure anticoagulation. Only subcutaneous arm veins were used as blood access points. The IVIG infusions (1g/kg) were scheduled as soon as possible after the plasma exchange session at 15 day intervals rather than on two consecutive days once a month (the usual timetable for treating autoimmune disorders), in the attempt to reduce the amount of infused IVIG removed weekly by the plasma exchange sessions. The infusions were slow, programmed to last at least 7-8 hours during which time the patients were opportunely hydrated. The following were considered contraindications to treatment: immunoglobulin A deficiency, renal failure and previous intolerance/allergy to IVIG. Low dose aspirin (100 mg/day), which was suspended a week before the planned delivery, was administered empirically to avoid IVIG's thrombophilia side effects.

Neonatal treatment. If the infant had even small amounts of serum IgA, normal kidney function, and positivity to maternal autoantibodies, IVIG treatment was begun as soon as possible after birth. IVIG infusions (1g/kg) were scheduled at 15-day intervals rather than on two consecutive days every month in the attempt to prevent blood viscosity and excessive plasma volume build-up in the neonates. The infusions were slow, lasting at least 10 hours during which time the infants were opportunely hydrated and carefully monitored. The IVIG sessions were generally carried out according to this timetable for four or five months and in any case until maternal antibodies were no longer detectable by ELISA assay. Just as in the case of neonatal thrombocytopenia due to passive autoantibodies from mothers with immune thrombocytopenia [27], due to the difficulty of finding peripheral blood access points and to neonatal stress provoked by multiple IVIG infusions, starting from February 2016 the IVIG treatment was reduced to one infusion of

0.8g/kg/day for two consecutive days, begun as soon as possible after birth. This timetable was utilized in the last four children (cases n. 13-16). In order to evaluate the uniformity of the study group treated with combined therapy the outcomes of the 12 children treated with the previous combined therapy were compared with those of the four children treated with the modified combined therapy.

The therapy for the control group

The treatment protocol for the control group referred exclusively to maternal therapy which consisted in most cases of oral dexamethasone or betamethasone (8 mg/day) administered alone for three consecutive days when CHB was detected, then reduced to 4 mg/day until delivery. In five cases a single IVIG infusion or plasma exchange sessions were prescribed in addition to fluorinated steroid therapy; these additions were performed without a definite plan. To evaluate the uniformity of the control group patients the clinical characteristics, antibody profiles and outcomes of the 14 mothers treated with steroids alone were compared with those of the five mothers treated with steroids+IVIG or steroids+plasma exchange. Unfortunately, due to the low number of cases treated, it was not possible to compare separately the patients treated with IVIG (n 3) and those with plasma exchange (n 2).

Pre and postnatal circulatory support

Fetuses with heart rates $\leq$ 50 beats/minute were generally treated with sympathomimetic drugs (e. g. Salbutamol), which increased the heart rate by 5–10 beats per minute for the remainder of gestation. In addition, these drugs were given to infants with a heart rate at birth $\leq$ 55 beats/minute who had signs of circulatory failure before putting on the pacemaker.

Autoantibody detection

Maternal serum samples were collected at the time CHB was detected; serum samples were collected from neonates at birth and before every IVIG infusion. Serum samples were stored at -80°C until anti-SSA/Ro and anti- SSB/La antibodies could be assayed using a home-made ELISA, following the method described by Klauninger et al. with minor modifications [28]. The cut-off values were calculated as the 99th percentile of results obtained by testing the sera of 100 healthy women matched for age with the patients in the study and control groups. The intra-and inter-assay coefficients of variation were <10% for both tests.

Statistical analysis

The results for categorical variables were expressed as frequencies and percentages; those for continuous variables were expressed as mean and standard deviation. Univariate analysis was performed to evaluate the association between the categorical variables using Fischer's exact test, and between the continuous variables using a nonparametric Mann–Whitney *U* test. A Kaplan–Meier survival analysis and the log-rank test were carried out to analyze the cumulative incidence of pacemaker implants in the infants in the two groups. A < 0.05 *p* value was considered significant. All statistical analyses were performed using GraphPad Prism statistical software (San Diego, CA, USA).

RESULTS

Thirty-five pregnant patients were enrolled between June 2000 and December 2019. Sixteen (the study group), all enrolled between January 2009 and December 2019, agreed to the maternal-neonatal combined therapy protocol. Nineteen patients (the control group), all enrolled between June 2000 and November 2015, were treated with other therapy protocols: 14 (73.7%) with fluorinated steroids alone, three (15.7%) with fluorinated steroids + one IVIG infusion, and two (10.5%) with fluorinated steroids + plasma exchange sessions, performed 17 and three times, respectively. The clinical characteristics and antibody profiles of the patients in the study group were not significantly different from those in the control group (Table 1). In particular the gestational age at the time CHB was detected and the length of time the children were followed after birth were not significantly different (Table 1). As can be seen in the supplemental Tables 1 and 2, the clinical characteristics, antibody profiles and outcomes of the 12 mothers and their children treated with the previous combined therapy were without significant differences with respect to those of the four mothers and their children treated with the modified combined therapy. The only significant difference observed was the duration of the post-natal follow-up, which was significantly shorter in the four children treated with the modified combined therapy because they were last enrolled in the study. Furthermore, the 14 cases treated with fluorinated steroids alone presented clinical characteristics, antibody profiles and outcomes without significant differences with those of the five cases treated with IVIG or plasma exchange in addition to fluorinated steroids (Supplemental Tables 3 and 4). These results taken as a whole allowed us to validly compare the study group with the controls. In addition, they led us to consider the modified combined

therapy as the current combined therapy also because it was better tolerated by both mothers and children.

The trends over time of CHB grade and of heart rate from the time it was detected to birth and to the end of post-natal follow-up and the outcomes of the combined-therapy children are reported in detail in Table 2. At birth, out of the seven cases in whom 2nd degree CHB had been detected, three (42.8%) regressed (in two to 1st degree and in one to normal sinus rhythm), three (42.8%) remained stable and one (14.3%) progressed to 3rd degree CHB. At the end of post-natal follow-up only two children (one 12 and the other 10 years old) had a stable 1st degree CHB. The other five progressed to a 3rd degree CHB. All nine children in whom a 3rd degree CHB was originally detected remained unchanged at birth and throughout the post-natal follow-up. Pacemakers were implanted in four of the infants (25%) during their first year of life; all had a 3rd degree CHB at detection and their heart rates ranged between 45 and 56 bpm. All (100%) the children treated with the combined therapy protocol are currently alive.

The trend over time of CHB degree and of heart rate from the time it was detected to birth and to the end of post-natal follow-up and the outcomes of the children treated with other therapies are illustrated in detail in Table 3. Out of the eight 2nd degree CHB registered at the time of detection, seven (87.5%) progressed to 3rd degree at birth. The remaining one, who was receiving steroid and plasma exchange treatment, continued to present 2nd degree CHB up until 27 months of age at which time the condition progressed to a 3rd degree block. Regression of CHB was not observed in any of the cases. Over a period ranging between 1 day and 48 months, pacemakers were implanted in 16 of the 18 live births (88.8%). Seventeen (89.5%) of the children treated with other therapies are currently alive including one undergoing cardiac transplantation, and two are dead.

The primary and secondary outcomes in the study and control infants are shown and compared in Table 4. Although the number of 2nd degree CHBs at the time it was detected was similar in the two groups, the progression of CHB from 2nd to 3rd grade at birth was significantly lower in the combined protocol group with respect to that in the group receiving other therapies. While there was no significant difference in heart rates recorded at the time of detection, average heart rates at birth were significantly higher in the infants treated with combined therapy with respect to those treated with other therapies (81.6 *vs* 57.3 beats/minute). In addition, at the end of the study the former had higher average heart rates than the latter (62.2 *vs* 46.0

beats/minute), but a statistical comparison could not be made due to the low number of infants without pacemakers in the control group. While there were no significant differences in the rates of dilated cardiomyopathy, defined as a pathologically large left ventricle or multiple chambers with an abnormally low left ventricle ejection fraction and of endocardial fibroelastosis, defined as abnormal areas of echogenicity on the endocardial surface of the cardiac chambers and/or valve leaflets [4] in the two groups, regression was noted only in the two infants belonging to the combined therapy group. Round hyperechogenic lesions of a 3.5 cm diameter on the posterior wall of the left atrium disappeared in one of these infants nine months after birth; in the other a mild hyperechogenicity of the tricuspid with mild valvular insufficiency were no longer detectable at birth. Three of the children treated with other therapies had cardiomyopathy/endocardial fibroelastosis, one of them suffered from a severe form of dilated myocardiopathy which required the implantation of a pacemaker at two days of life and a heart transplant at 17 months; the second had dilated cardiomyopathy and underwent pacemaker implantation at 23 days and suffered sudden death at 21 months; the third had diffuse hyperechogenicity, valvular thickening, biventricular dilation and pericardial effusion and underwent pacemaker implantation at 7 days of life and now, 5 years later, is alive but suffers from mental retardation. The number of pacemakers implanted in the children receiving combined therapy was significantly lower than that in the children on other therapies. In addition to confirming a significant difference between the two groups, the Kaplan Meier curve (Figure 1) showed that the pacemakers were implanted within the first month of life only in the children treated with other therapies (six cases). There were three negative outcomes, all occurring in children treated with other therapies: one death at 29 weeks of gestation, one sudden death at 21 months of life and a heart transplant at 17 months of age. There were no significant differences in the secondary outcomes between the two groups with the exception of the Apgar score at 5 minutes, which was significantly higher in the children treated with other therapies.

According to the responses to the questionnaires filled out by the mothers of the children treated with combined therapy, all the children, including those with pacemakers, had a good quality of life with a good ability to adapt to efforts. Their mean weight percentiles were 32.9 ± 30.8 SD, range 3-95 and mean height percentiles 46.1 ± 28.4 SD, range 3-91. All were progressing normally at school. Two of them (12.5%) had celiac disease and one (6.2%) had learning disabilities. The frequency of these disorders in the study group is

comparable to that reported in the general pediatric population [29, 30]. Regarding steroid side-effects, while the long-term neurodevelopmental impairments in CHB children with prenatal exposure to steroids remains an object of controversy [31], oligohydramnios and IUGR would potentially be related to use of high-dose glucocorticoids during pregnancy [8]. These pregnancy complications were observed in nine (56.2%) patients on combined therapy (6 IUGR, 2 oligohydramnios and 1 IUGR + oligohydramnios) and in six (31.6%) control patients (4 IUGR and 2 IUGR + oligohydramnios) without significant difference ($p = 0.1821$). No important side effects linked to the use of plasma exchange or IVIG were registered in the mothers, fetuses, or neonates.

DISCUSSION

This study reports on one of the few treatments for CHB that exists besides fluorinated steroids alone and it compares the short and long-term outcomes of a combined maternal-neonatal therapy protocol with those obtained using other therapies. It is important to point out immediately that the outcomes of the combined therapy protocol were compared primarily with those of fluorinated steroids alone (73.7% of cases) because use of fluorinated steroids together with IVIG or plasma exchange was limited to only five patients (26.3%) who were treated without a definite plan. Nevertheless, the children on combined therapy showed a significantly lower progression from 2^{nd} to 3^{rd} degree block at birth, a significant increase in heart rate at birth and a significantly lower number of pacemaker implants during the follow-up period with respect to the children treated with the other therapies. Although the differences were not significant, regression of 2^{nd} degree blocks were noted only in three of the children in the combined protocol group; 1st degree blocks currently persist in two of them 12 and 10 years later. It is interesting to note that according to the Italian Registry on Immune-Mediated CHB, three of the five (60%) 2^{nd} degree CHB regressions occurred in cases treated with the combined maternal-neonatal therapy and the other two (40%) occurred in patients treated with dexamethasone plus plasma exchange and dexamethasone alone. [32]. Moreover, the three negative outcomes (one intrauterine death, one sudden death at 21 months, and a heart transplant at 17 months) occurred exclusively in the control group.

It can be speculated that the benefits associated to the combined therapy protocol are gained by a summation of the effects of the three therapies put together according to a logic based on the reasonable

pathogenetic mechanism of autoimmune CHB [2]. First of all, betamethasone appears to reduce inflammation of the fetal conduction tissue and myocardium. Second, as has been demonstrated [26], plasma exchange removes large quantities of offending autoantibodies from the maternal–fetal circulation, and last IVIG seems to be able to neutralize the effect of those autoantibodies still present after plasma exchange [33]. In fact, used singularly, steroids [8], plasma exchange [34] and IVIG [35] do not seem to be able to prevent progression of 2nd degree to 3rd degree CHB. According to one report, a 2nd degree block regressed to a normal sinus rhythm when fluorinated steroids were used in association with IVIG within 12 h of the time an abnormal fetal heart rhythm was registered by a home surveillance monitor and confirmed by echocardiogram. In another case, in which there was a 24-h delay in the home monitoring-diagnosis-treatment window an irreversible progression to 3rd degree CHB took place [12]. In our experience, the treatment of two 2nd degree CHB with combined therapy after one and three days, respectively from echocardiographic detection, led to a stable regression to 1st degree. Thus, it could be hypothesized that by removing large quantities of maternal autoantibodies, plasmapheresis is able to counteract the autoantibodies' harmful effect on the fetuses' cardiac conduction tissue even after 72 hours from CHB detection.

One of the study's most salient findings was that the efficacy of combined therapy was registered only in cases of 2nd degree blocks, thus, we could deduce that an improvement in the CHB condition is possible only in the early stages of injury when inflammation in the atrioventricular node and myocardium is still reversible and before the tissues have become fibrotic and calcific [36]. The efficacy of the combined therapy protocol seems thus strictly tied to the early detection of an incomplete CHB and rapid treatment onset. These data agree with the findings of some studies demonstrating that a complete block can occur in even less than 24 hours from the time that a normal sinus rhythm is registered [6, 37]. On the basis of these observations, pregnant women positive to anti-SSA/Ro and/or anti-SSB/La antibodies should routinely undergo at least weekly echocardiographic monitoring between the 18th and 26th weeks of gestation and, if possible, take twice daily home fetal heart rate and rhythm readings [12]. In fact, the two children who regressed to a persistent 1st degree CHB were treated earlier (1 and 3 days after a 2nd degree CHB was detected) than the other five children with 2nd degree CHB who at birth showed one temporary regression to 1st degree CHB, three non-progression to 3rd degree CHB, and one progression to 3rd degree block.

It has yet to be demonstrated that combined therapy can be effective in the event of 3rd degree CHB. It is possible that blocking the progression of endomyocardial injury improves cardiac contractility and increases the basal heart rate, thus, delaying the need for pacemaker implantation as well as its potential complications [4, 9]. In fact, at the end of the post-natal follow-up, 10 (71.4%) out of the 14 children with a 3rd degree block did not require a pacemaker, had a normal left ventricular ejection fraction and are leading normal lives.

It is placental transfer of maternal anti-Ro/La antibodies which induces inflammation of the fetal myocardial specialized conductive tissues and surrounding myocardium and leads to CHB. However, 16-20% of affected fetuses can develop more diffuse endomyocardial disease manifested as cardiomyopathy usually associated with endocardial fibroelastosis [3, 9]. The prognosis for fetuses and infants with these complications is generally poor, with death or need for cardiac transplantation in 85% of cases despite pacemaker therapy [3]. In the cases studied here, the two fetuses receiving combined treatment showing echocardiographic signs of endocardial fibroelastosis had regression of the lesions, one at birth and one nine months later. The two children treated with fluorinated steroids alone and the third one with fluorinated steroids + one session of IVIG, had instead, unfavorable outcomes. Although the comparison did not produce a statistically significant result, these findings seem to support the hypothesis that in addition to reducing the inflammation of the conducting tissue, the combined therapy protocol may also diminish the immune-mediated cardiac inflammation provoked by maternal autoantibodies and indirectly improve cardiac contractility. To note, some investigators have reported a resolution of the signs of myocarditis or an improvement in the outcomes of fetuses affected with cardiomyopathy/endocardial fibroelastosis when fluorinated steroids and IVIG courses were utilized. [3, 38].

The idea of administering IVIG treatment to neonates after birth was based on reports describing a risk of progression to a more severe heart block and/or occurrence of other cardiac complications even after birth [8, 39, 40]. Both in 12 babies previously treated for a longer time with a greater number of IVIG infusions and in four treated with two IVIG infusions only, the degree of CHB and heart rate remained unchanged during the five months in which maternal autoantibodies were still detectable in the blood of children. As has been verified by other studies [41,42], plasma exchange during pregnancy never caused noteworthy side effects. Also IVIG infusions both in mothers and neonates were well tolerated.

As far as study limitations are concerned, given the rarity of CHB cases, there were only a limited number of study and control patients. Furthermore, the fact that the women and their children in the control group were monitored several years earlier than those in the study group may have introduced potential confounding variables in their management. In particular, also due to the lack of well-defined guidelines concerning the indications for pacing children with autoimmune CHB, it is possible that in this study there was a selection bias towards pacemaker implantation; e.g. in three infants on combined therapy with heart rate at birth ≤55 beats/minute, the pacemaker implantation was postponed of 10, 5 and 12 months, respectively, compared to the five control infants treated with other therapies who put on the pacemaker in the first month of life despite having the same heart rate. However, the homogeneity of the women's clinical characteristics, antibody profiles, gestational ages at CHB detection and durations of post-natal follow-up (Table 1) increases the reliability of our data. Finally, if the high cost of the combined strategy protocol can be considered another study limitation, the expense and energy involved in implementing this therapy could be justified by the small number, given the rarity of the condition, of patients requiring it.

In conclusion, the combined therapy protocol outlined here based on maternal treatment of daily fluorinated steroids, weekly plasma exchange and fortnightly IVIG infusions plus neonatal treatment with IVIG produced better short and long-term outcomes in infants with CHB than in those receiving other therapies (especially fluorinated steroids alone). The encouraging results of this small prospective study need to be verified by larger multicenter controlled studies which will be able to establish if the protocol can efficaciously treat this rare but devastating disease.

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ADDITIONAL INFORMATION SECTION

Conflict of interest The authors declare no competing interests.

Author contributions AR developed the project, and wrote the manuscript; AC performed echocardiograms

in fetuses and in children during the post-natal follow-up and analyzed the data; MT performed laboratory assays and analyzed the data; MF, TDR, AC, MZ, CG, AH acquired data, and played an important role in interpreting the results; GDS critically reviewed the manuscript. All authors reviewed the manuscript for important intellectual contents and approved the final version. All authors agree to be accountable for all aspects of the work.

Data availability Correspondence and requests for original data should be addressed to A.R.

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Figure legends

Fig. 1 Kaplan–Meier survival analysis and the log-rank test revealed that the cumulative incidence of pacemaker implants in the children with congenital heart block treated with combined maternal-neonatal therapy (**C**) was significantly lower than in those treated with other therapies (**O**).

Table 1. The clinical characteristics and antibody profiles of the women in the study group who were treated with the combined therapy protocol and those in the control group who were treated with other therapies.

	Combined therapy (n 16)	Other therapies[a] (n 19)	*p* value
Maternal age, mean years (SD)	33.3 (4.7)	31.4 (4.1)	0.2606
Maternal ethnicity, n (%)			
Italian	13 (81.3)	17 (89.5)	0.6418
other	3 (18.7)	2 (10.5)	0.6418
Maternal diseases, n (%)			
Sjögren'syndrome	6 (37.5)	4 (21.1)	0.4543
Undifferentiated connective tissue disease	3 (18.7)	7 (36.8)	0.2853
Systemic lupus erythematosus	0	1 (5.3)	1.0000
No (asymptomatics)	7 (43.7)	7 (36.8)	0.7391
Maternal antibody positivity, n (%)			
Anti-SSA/Ro	5 (31.2)	8 (42.1)	0.7267
Anti-SSB/La	0	0	-
Anti-SSA/Ro+Anti-SSB/La	11 (68.7)	11 (57.9)	0.7267
Time at CHB detection, mean weeks (SD)	23.7 (4.2)	23.0 (2.2)	0.7628
Post-natal follow-up time, mean weeks (SD)	7.4 (3.2)	6.2 (4.5)	0.1230

n number, SD standard deviation, CHB congenital heart block.

[a]14 were treated with fluorinated steroids alone, 3 with fluorinated steroids+intravenous immunoglobulins and 2 with fluorinated steroids+plasma exchange.

552 **Table 2.** The pattern over time of CHB grade and mean heart rate and the outcome in the children treated
553 with combined therapy.

Case number	At detection grade (mean bpm)	At birth grade (mean bpm)	Follow-up years	Currently grade (mean bpm)	Outcome
1	2nd (74)	1st (158)	12	1st (99)	alive
2	3rd (52)	3rd (52)	11	3rd (PM, 10 months)	alive
3	2nd (80)	1st (130)	10	3rd (62)	alive
4	3rd (63)	3rd (85)	10	3rd (50)	alive
5	3rd (60)	3rd (58)	10	3rd (44)	alive
6	2nd (74)	normal sinus rhythm (135)	10	1st (84)	alive
7	2nd (80)	2nd (80)	8	3rd (55)	alive
8	2nd (67)	2nd (70)	8	3rd (55)	alive
9	2nd (70)	2nd (77)	8	3rd (53)	alive
10	3rd (45)	3rd (44)	8	3rd (PM, 5 months)	alive
11	3rd (65)	3rd (73)	7	3rd (48)	alive
12	3rd (56)	3rd (58)	5	3rd (PM, 6 months)	alive
13	3rd (60)	3rd (65)	5	3rd (62)	alive
14	3rd (55)	3rd (55)	3	3rd (PM, 12 months)	alive
15	2nd (65)	3rd (85)	2	3rd (85)	alive
16	3rd (85)	3rd (80)	1	3rd (50)	alive

554 bpm beats per minute, PM pacemaker.

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Table 3. The pattern over time of CHB grade and mean heart rate and the outcome in the children treated with other therapies.

Case number	Other therapies	At detection grade (mean bpm)	At birth grade (mean bpm)	Follow-up yrs	Currently grade (mean bpm)	Outcome
1	FS	3rd (45)	3rd (45)	2	3rd (PM, 12 days)	alive
2	FS	2nd (70)	3rd (80)	6	3rd (PM, 11 months)	alive
3	FS	3rd (62)	3rd (70)	7	3rd (PM, 3 months)	alive
4	FS	3rd (50)	3rd (65)[a]	2	3rd (PM, 2 days)	transplant 17 m
5	FS	2nd (60)	3rd (55)	3	3rd (PM, 1 day)	alive
6	FS	3rd (55)	3rd (50)	7	3rd (PM, 2 months)	alive
7	FS	3rd (55)	3rd (50)	1	3rd (PM,1 day)	alive
8	FS	3rd (62)	3rd (62)	6	3rd (PM, 2 months)	alive
9	FS	3rd (45)	3rd (45)	5	3rd (PM, 35 days)	alive
10	FS	3rd (52)	3rd (50)	6	3rd (PM, 42 days)	alive
11	FS	2nd (66)	3rd (53)	2	3rd (PM, 23 days)	dead 21 m
12	FS	3rd (45)	3rd (50)	9	3rd (40)	alive
13	FS	2nd (86)	3rd (85)	9	3rd (52)	alive
14	FS	3rd (62)	3rd (50)	6	3rd (PM, 48 months)	alive
15	FS +IVIG	2nd (80)	3rd (80)	3	3rd (PM, 17 months)	alive
16	FS +IVIG	2nd (60)	NA	NA	NA	fetal death 29 w
17	FS +IVIG	3rd (48)	3rd (40)	5	3rd (PM, 7 days)	alive
18	FS +PE	2nd (64)	2nd (60)	19	3rd (PM, 48 months)	alive
19	FS +PE	2nd (50)	3rd (47)	14	3rd (PM, 11 months)	alive

bpm beats per minute, yrs years, PM pacemaker, FS fluorinate steroids, m months, IVIG intravenous immunoglobulins, NA not applicable due to fetal death, w weeks of gestation, PE plasma exchange.

[a] newborn with dilated cardiomyopathy

Table 4. The outcomes of the children in the two groups: one treated with combined therapy and the other with other therapies.

	Combined therapy (n 16)	Other therapies[a] (n 19)	*p* value
Primary outcomes			
2nd degree CHB at detection, n (%)	7 (43.7)	8 (42.1)	1.0000
regression at birth, n (%)	3 (42.8)	0	0.0769
progression at birth, n (%)	1 (14.3)	7 (87.5)	0.0101[b]
non-progression at birth, n (%)	3 (42.8%)	1 (12.5)	0.2821
3rd degree CHB at detection, n (%)	9 (56.2)	11 (61.1)[c]	1.0000
regression, n (%)	0	0	-
Mean heart rate			
intrauterine, mean bpm (SD)	65.7 (11.1)	58.8 (11.4)	0.0631
at birth, mean bpm (SD)	81.6 (32.3)	57.3 (13.6)	0.0070[b]
currently, mean bpm (SD)[c]	62.2 (17.5)	46.0 (8.5)	NA
Cardiomyopathy/endocardial fibroelastosis, n (%)	2 (12.5)	3 (15.8)	1.0000
regression, n (%)	2 (100)	0	0.1000
progression, n (%)	0	3 (100)	0.1000
Pacemaker implantation, n (%)	4 (25%)	16 (88.9)[d]	0.0003[b]
Negative outcomes[e], n (%)	0	3 (15.8)	0.2336
Secondary outcomes			
Pregnancy complications[f], n (%)	10 (62.5)	7 (36.8)	0.1811
Gestational age at delivery, mean weeks (SD)	34.5 (1.8)	35 (2.7)	0.3731
Birth weight, mean percentiles (SD)	17.1 (13.7)	24.8 (18.9)	0.2987
Apgar score at 5 minutes, mean (SD)	8.2 (0.9)	9.0 (0.6)	0.0141[b]

n number, bpm beats per minute, SD standard deviation, NA comparison not applicable for low number of children on other therapies without pacemakers. [a]14 were treated with fluorinated steroids alone, 3 with fluorinated steroids+intravenous immunoglobulins and 2 with fluorinated steroids+plasma exchange. [b]Significant value ($p<0.05$). [c] current heart rate was evaluated only in children without pacemaker. [d] Due to one intrauterine death at 29 weeks, the percentage is calculated on 18 cases. [e]Including one intrauterine death at 29 weeks, one heart transplant at 17 months and one sudden death at 21 months. [f]Including intrauterine growth restriction, premature rupture of membranes, abruptio placentae, intrauterine death, oligohydramnios, anhydramnios and hypertension.