

# FROM SPLENIC ANEMIA IN INFANCY TO MICROCYTHEMIA. THE ITALIAN CONTRIBUTION TO THE DESCRIPTION OF THE GENETIC BASES OF THALASSEMIA

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## Summary

This article traces out part of the history of studies of the genetic bases of thalassemia carried out in Italy. In particular it illustrates the research and discussions that between the late 1920s and the second half of the 1940s led to the description of the genetic basis of beta-thalassemia. The article also discusses the theoretical and methodological difficulties encountered by Italian research, explaining why, despite the large number of thalassemia cases and data collected for this disease, Italian researchers succeeded in demonstrating its Mendelian transmission only at the same time as the US researchers.

The exact demonstration of the Mendelian transmission of beta-thalassemia<sup>1</sup> is the result of research carried out between 1940 and 1947 independently in the United States and Italy. Probably for linguistic reasons, literature on the history of thalassemia, with the works of David Wheaterall<sup>2</sup> and Maxwell Wintrobe<sup>3</sup> in the forefront, has extensively documented the contribution made by US research and displays only a partial knowledge of the research and debate on the genetics of Cooley's disease that had been conducted to a certain extent in Italy ever since the 1920s. The present article aims to contribute to bridging this persistent historiographic gap.

## Familiarity as the principal note and the first anamnestic investigations

One first observation and analysis concerning the hereditary factor in Cooley's disease type syndromes was published in 1928 by Luigi Auricchio. He described 12 cases of splenic anemia in children aged from several days to 5 years from 5 different families. The interest of his study lies in the fact that for the first time an extensive study was made of the families of the sick children. As Auricchio wrote:

"Interest in the cases we have briefly illustrated lies not in the clinical-hematological syndrome, which largely fits the common pattern of splenic anemia of infancy, but rather in the repetition of the syndrome itself in many or all of the children born of the same parents. And it was indeed to report the familial nature of this serious form of infantile hemopathy and in view of the utility of making some considerations on the relative problem of its etiopathogenesis that we deemed it of some interest to draw attention to them."<sup>4</sup>

Auricchio expressed the hope that:

"anamnestic investigation would be extended more rigorously and methodically in all cases of apparently idiopathic blood diseases of obscure origin affecting children in the same family in order to detect any hereditary pathogenic influences"<sup>5</sup>

However, Auricchio's suggestion largely went unheeded.

In 1932 Luigi Cerza, one of Rocco Jemma's pupils at the pediatric clinic of Naples University, confirmed the familial nature of the disease by studying the case histories of nine families, five of which already studied by Auricchio in 1928, which had been selected owing to the presence of children with a generic diagnosis of anemia accompanied by splenomegaly<sup>6</sup>. The investigations carried out on family members were however rather unsystematic, and the identification of the cases of actual disease uncertain

and based on confused diagnostic criteria. No mention was made of Cooley's disease, which Cerza in all likelihood was not familiar with, like a large number of his Italian and European colleagues of the time. Many of the symptoms and clinical signs described nevertheless seem to point to a series of cases of thalassemia: in some families, the death of several children from anemia accompanied by splenomegaly, erythroblastosis and other red corpuscle morphology alterations.

Of interest in Cerza's work is a discussion of Mendel's laws in medicine, which was quite rare in medical literature, especially Italian, at the time<sup>7</sup>. This formed the premise for a discussion of the significance of the occurrence of the disease in several generations and of the side effects observed in several families in these case histories. However, Cerza did not subject his data to any mathematical treatment, also because of the uncertain nature of the data themselves, which were the result of superficial clinical observations, unsystematic hematologic research and simple anamnestic tests, nor did he try to build up a genealogical picture of the form of hereditary transmission postulated. However, he felt confident enough to suggest a hypothesis concerning the disease's mode of hereditary transmission:

"we can thus say that familial type splenic anemias in young children may be considered to be caused by recessive hereditary factors that are transmissible to the members of the same family but come from individuals from a different family (heterozygotes) [...]"<sup>8</sup>

He too, like Auricchio, appealed to researchers

"to gather further observations and subject them to detailed anamnestic investigation, supplementing them with the results of clinical and biological research [...] for the purpose, on the basis of proven data, of including these blood diseases among hereditary disorders of a recessive nature"<sup>9</sup>

Cerza's invitation was to remain a dead letter for many years to come.

## **First studies on twins**

In a long paper published in 1936 in the pediatric journal *Il Lattante*, Marino Ortolani discussed the familial and congenital nature of Cooley's disease, illustrating the case of two monozygotic twin girls that had been brought to his attention a few days after their birth<sup>10</sup>. The study contains a detail drawn from the diaries containing the results of the clinical investigations. Hematological and radiological research clearly pointed to Cooley's disease. As did the results of the anamnestic investigations as far as the formulation of a hypothesis to account for the hereditary transmission mechanisms are concerned. Both the mother and the father of the sick twins displayed the slanting eyes and skull deformations typical of Cooley's disease. However, Ortolani did not carry out any blood tests or X-ray examinations, which shows the scant consideration in which the matter was held or else the incapacity to grasp the significance.

A fresh report on Cooley's disease in twins was published in 1940 by Giovanni Careddu<sup>11</sup>. Compared with Ortolani's paper, Careddu introduced some interesting new elements related to familiarity. He observed the presence of a significant hepatosplenomegaly and hemolytic jaundice in the mother. According to Careddu this observation:

"apparently demonstrates that, even in the absence of direct hereditariness of the disease, the parents, who are apparently healthy and with a normal blood test, display several of the signs that are part of the disease itself."<sup>12</sup>

He failed however to proceed beyond this simple observation or to use it as the basis for fresh research on the topic.

## **Theoretical and methodological problems related to early research on the genetics of thalassemias**

Between the late 1930s and the early 1940s, the familial nature of Cooley-like syndromes was repeatedly observed and reported in Italy<sup>13</sup>. The observation of familiarity was however never followed by rational investigations or carried out in accordance with the principles and using the tools of genetic analysis. For instance, genealogical reconstructions were practically non-existent. Also absent from this literature is any attempt to classify the objects of the observation, to isolate any quantifiable characters that might lend themselves to mathematical treatment. This explains the multifarious and empirical observations of familial traits, now the morphological features of the face, now certain hematological peculiarities, now radiological observation, now also hyperhemolysis, and so on. This is due to the persistent disagreement in the Italian scientific community concerning the actual identity of Cooley's disease and, to an even greater extent,

concerning the nature of the various Cooley-like syndromes observed in cases of Mediterranean anemia in adults or in Cooley related traits observed in apparently healthy parents.

Also as regards the analysis of familiarity, these studies tended above all to address issues of pathogenesis and were obviously restricted to the conceptual domain of clinical practice, having the primary, if not exclusive, objective, of identifying a rational and efficacious treatment.

The uncertain nosographic classification of the case histories discussed and the incomplete acceptance of Cooley's classification ultimately led to a barren conflict between quite similar data (microcytosis, increased globular resistance, skeletal alterations), in particular with regard to the issue of the origin of hyperhemolysis. This was probably due to the fact that, amid the confusion and nosological uncertainty, and owing to a complete ignorance of the causes, researchers continued to refer to hemolytic jaundice, the clinical entity that best characterized the most obvious symptom, or else referred to certain of its particular aspects. One illuminating example of this is the study published by Usseglio and Massobrio in 1934 in which they discussed cases involving relatives of patients who displayed very slight or no signs of hyperemolysis but were nevertheless classified as carriers of constitutional hyperhemolysis<sup>14</sup>.

The lack of familiarity with specifically medical genetics at the time was also an obstacle to progressing beyond vague conceptualizations of the familial character as an idea of constitutional factor or sign. Owing to its theoretical nature, this notion proved unassailable by any attempt at analytical treatment, or reduction to quantitative and univocally relatable elements. This state of affairs can thus provide a historical explanation of the disconcerting statements made by Micheli, Penati and Momigliano Levi at Pavia during the meeting of 23 June 1935 of the Italian Hematology Association and the consequent address given by Adolfo Ferrata. After accurately describing in 5 cases the hematological peculiarities of the microcythemic conditions and postulating that under this disease was "a constitutional alteration of the bone marrow", the three went on to discuss a case of Cooley's disease in a little Sardinian girl and the research carried out on the familial side. These studies had brought to light in the parents the hematological traits described in the first case history, which were similar in type if not in severity to those of the child. Instead of evaluating the nature of these clear-cut relationships and striking similarities, the three authors observed that:

"Disregarding the curious observed presence in both parents of our patient of the familiar morphological signs reported, a coincidence of which moreover we can only take note, it appears quite clear that a close analogy exists between these findings and those typical of the forms that we are investigating"<sup>15</sup>

In the discussion that followed the communication delivered by the three, Adolfo Ferrata, lecturer in clinical medicine at Pavia and founder of the journal *Haematologica*, seemed even less inclined to interpret the observed facts in terms of genetic explanations and claimed that "The hereditary constitutional factor seems to be demonstrated by the coexistence of lesions in both parents and children. However, it is odd that both parents had it and this leads me to believe there is an environmental factor"<sup>16</sup>.

## **Beyond anamnestic observations: the research of Valentino Angelini**

The first systematic research aimed at demonstrating the hereditary nature of the condition was carried out at the pediatric clinic of the University of Padua by Valentino Angelini in 1937. Angelini declared he was surprised that

"precisely in a form of anemia in which the constitutional attribute emerges so clearly in the familial onset of the disorder, the systematic study of the family members should have been overlooked: such a study would have illuminated us more clearly regarding the true existence of a transmissible diathesis."<sup>17</sup>

Angelini proceeded at last beyond mere anamnestic research and subjected the patients' relatives to minute clinical and hematological investigation. In particular he investigated the behaviour of globular resistance and bilirubinemia, the signs that more than any others had been observed in the study of Cooley's disease and similar disorders. His investigation involved six families for a total of 26 individuals. This is how Angelini reported the data obtained: "The apparently healthy parents of children affected by Cooley's anemia had [...] a globular resistance behaviour that was characterized by an increased maximum globular resistance [...]. The apparently healthy siblings of children affected by Cooley's anemia as a general rule also displayed an increase in the maximum resistance"<sup>18</sup>. Angelini also demonstrated that each member of the family displayed hyperhemolysis, "just as happens in Cooley's disease"<sup>19</sup>

By isolating the two quantifiable and comparable hematological signs and by investigating the behaviour of entire families, Angelini had imposed a turning point on research into the hereditary transmission of Cooley's disease. The data he could draw on led to decisive developments in investigations

to characterize the genetics of the disease. Once again, however, the lack of familiarity with genetics, which is denoted by the complete absence of any reference to possible hereditary mechanisms and of genealogical reconstructions, allowed a rough and ready clinical approach aimed at demonstrating the presence of a familial diathesis, a hyperhemolytic constitution, to prevail:

“in certain family strains, these anomalies seem to represent hematological and biological signs of a single diathesis of the hyperhemolytic type: in some subjects this diathesis attains overt expression in a serious morbid form (Cooley’s anemia), while in other members, who remain healthy, it remains in a form that can be detected by laboratory tests.”<sup>20</sup>

Another peculiarity in Angelini’s work is its extreme neglect of biological consistency in accounting for the observed phenomena, for instance, increased globular resistance, to which he attributed the same significance and the same role as its decrease in constitutional hemolytic jaundice and above all the fact of not being curious about or investigating the possible links between what were considered conflicting conditions, such as increased globular resistance and hyperhemolytic diathesis.

The absolute predominance of the hyperhemolytic doctrine eloquently expressed in the interpretation that Angelini gave of his interesting results was reiterated also in the discussions that two years later Frontali and Rasi devoted to Angelini’s research in a long review paper on the topic<sup>21</sup>. The two claimed that in Cooley’s disease it was not a question of “the hereditary transmission of a particular mutation of the erythrocyte but rather of a peculiar *hyperhemolytic diathesis*”<sup>22</sup>. And to support this thesis they claimed to have found an ultrafilterable factor favouring hemolysis in 8 out of 9 cases.

### **Heterozygote-stigmata/Homozygote-Cooley’s disease. Ignazio Gatto**

An important contribution to the understanding of the genetics of thalassemia in Italy was ultimately (between 1941 and 1942) to come from the studies of Ignazio Gatto. At the pediatric clinic of Palermo University, even before 1940, he had worked on a substantial number of Cooley’s disease case histories. Twenty-four cases had been reported in a communication to the XVII Italian Pediatric Congress examining the familiarity of the condition (9 cases)<sup>23</sup>. In addition Gatto observed that in the five families in which the disease was found in more than one member, the cases observed differed in seriousness and course. The data led Gatto to undertake research on the parents and relatives of the sick children.

Gatto’s investigations focused on eight families in which at least one individual suffered from Cooley’s disease, for a total of 26 individuals<sup>24</sup>. In 5 families more than one case was present; in one family the disease was present in a maternal and paternal collateral line. On the hematological side, “the most constant and characteristic alterations were due to the presence of microcytes and increased maximum globular resistance”. Microcytosis and increase globular resistance were found in 25 out of a total of 26 subjects involved in the study and in one family also in two successive generations prior to that of the sick individual. Gatto stressed that the most significant element in these observations was that the alterations constantly identified appeared in both parents. The phenomenon affected also certain anatomical traits, such as the width and prominence of the cheekbones, and some radiological evidence, such as the greater transparency of the skull, which to some extent recalled the initial stages of the so-called “*cranio a spazzola*” (brush skull) typical of Cooley patients.

This complex of results therefore forcefully suggested the presence of a hereditary condition. As Gatto wrote: “The frequent or constant existence, as in my cases, of stigmata in both parents, seems to point to a dominant type with a lethal homozygotic effect”<sup>25</sup>. What struck Gatto was in particular the regular finding of microcytosis and increased globular resistance, signs of the illness evidently possessed at the heterozygotic state by carriers, a dominant hereditary character as a result of which “the onset of the disease is thus possible only when the necessary homozygotic combinations occur”<sup>26</sup>. Gatto was thus the first to explicitly propose the heterozygote-stigmata and homozygote-Cooley’s disease equation.

### **From the characterization of the early hypotheses on the genetic causes of the clinical variability of thalassemia: Ezio Silvestroni and Ida Bianco**

The year in which Gatto published the hypothesis that Mediterranean anemia was a homozygotic condition of a genetic anomaly expressed in the form of microcytosis and increased globular resistance, at the medical clinic of the University of Rome, Ezio Silvestroni and Ida Bianco chanced to approach studies on Cooley’s disease. This took place during studies to verify the hypothesis discussed at the time in the

literature, namely that the red corpuscles of cancer patients were characterized by increased fragility in slightly hypotonic solutions and vice versa a decrease in more dilute solutions. Begun in early 1942, these studies involving 50 cancer patients and 50 control subjects, did not reveal differences in red corpuscle behaviour in a series of solutions of increasing concentration. Silvestroni and Bianco nevertheless found among the control subjects 4 individuals characterized by a strong increase in osmotic globular resistance, and postulated that “such a strong increase in the peak resistance represented a hematological sign of familiarity”<sup>27</sup>.

On the basis of the observation of this anomaly the two researchers examined 400 healthy subjects, some recruited from among the staff at the general medicine clinic of Rome University who had already been tested in the preceding study and partly among the relatives of patients admitted to the clinic and among the evacuees who flocked into the outpatients department every day.

#### *The characterization of microcythemia: 1943-1945*

Of these 400 individuals 7 were found to be carriers of a permanent increase in globular resistance. Even more significant was the fact that the 7 subjects displaying increased osmotic globular resistance were also carriers of hyperglobulia, hypochromy, microcytosis and altered erythrocyte morphology. On 26 November 1943 the two researchers presented their case histories to the Rome Medical Academy<sup>28</sup>, reporting the dominant hereditary character:

“indeed in 3 of the 7 subjects with increased globular resistance, we traced the mode of transmission of this character in the descendents, finding that when one of the parents was affected by this anomaly, only some of the children were immune while the others were affected by it.”<sup>29</sup>.

In this connection Silvestroni and Bianco reconstructed the family trees of the subjects studied and were thus also able to observe that “this anomaly has no sex-related preferences and is always complete, that is, as we have seen, it consists of increased globular resistance, hyperglobulia, hypochromy and true microcytosis”<sup>30</sup>. In this sense, Silvestroni and Bianco had immediately distinctly characterized the complex of clinical signs of the condition that would later be called microcythemia<sup>31</sup>. This is in contrast with the confusion and disagreements regarding the characteristic findings considered in investigations of Cooley-like syndromes that marked practically all the research described hitherto.

It is also worth mentioning that right from this first publication on the topic, Silvestroni and Bianco were aware of the exact correspondence between the geographic origin of the carriers observed by them with the now well-known one of Cooley’s disease patients.

#### *Towards an understanding of the hereditary mechanisms*

In their next two works, the first presented on 24 December 1944 to the Rome Medical Academy<sup>32</sup> and the second published only in 1946 as a result of causes due to the war<sup>33</sup>, the two researchers published the results obtained for the new case histories of microcytic anemia obtained from investigations involving a further 1100 persons. Starting in 1943 they had examined a total of 1500 persons and identified 31 carriers bearing the anomaly to which they had given the name of constitutional microcytic anemia (intermediate thalassemia in current nomenclature). With the inclusion of relatives, the number of carriers observed rose to 88. This study confirmed the idea of the hereditary nature of the anomaly as a Mendelian character and indicated that the incidence of the anomaly was higher among subjects originating from Sicily, Sardinia and Campania.

The years that followed coincided with a period of extremely intense study and numerous publications. In the meantime, in a short communication delivered to the Rome Medical Academy on 29 December 1945 the two researchers introduced some important conceptual distinctions into the broad and continuous spectrum of clinical signs which was in any case linked to the complex of hematological anomalies described by them<sup>34</sup>. The hematological anomaly was called microcythemia. In the broad clinical spectrum of its manifestation microcythemia might therefore not be expressed, as in the subjects in the case histories that displayed no symptoms, or else give rise to constitutional microcytic anemia. As a result, starting from the observed analogies between constitutional microcytic anemia and Cooley’s disease, the two researchers asked themselves whether microcythemia was in some way linked to Mediterranean anemia itself. To this end, in 1945 they examined the parents and other relatives of 26 children diagnosed with Cooley’s anemia, finding that microcythemia was present in one or both parents<sup>35</sup> (Silvestroni and Bianco, 1945-46, 1946a and 1946-47a). Silvestroni and Bianco also studied the parents of 6 children diagnosed with Jaksch’s pseudoleukemic anemia, finding microcythemia in the parents of the only two children among the 6 who died<sup>36</sup>. Lastly, they pointed out that the families of Cooley patients came from the same geographic areas as those in which the presence of microcythemia had been observed.

In 1946 the two researchers postulated that the origin of microcythemia should be sought “in a mutation (à la De Vries) of the erythropoietic system, probably occurring in a human strain of the great family of Mediterranean peoples”<sup>37</sup>. Evidence to this effect, according to Silvestroni and Bianco, is the “fact that only among the inhabitants of the Mediterranean basin have such diseases been described, namely Cooley’s disease and constitutional microcytic anemia, which stem from this anomaly”<sup>38</sup>.

In 1946 Silvestroni and Bianco examined a further 53 subjects who were carriers of microcythemia, the majority of whom belonged to 12 family strains and the others were isolated subjects or pairs of siblings. That year the two researchers had already studied a total of 224 microcythemic subjects, the majority of whom belonging to 54 family nuclei<sup>39</sup>. On the basis of anamnestic and hematological investigations, the two researchers compiled the family trees of the 12 families.

### *Apparent anomalies in the Mendelian hypothesis*

The overall results were quite similar to those obtained by the Greek researcher Jean Caminopetros<sup>40</sup> who, like Silvestroni and Bianco, had observed increased resistance more frequently in only one and less frequently in both parents of the sick subjects; they differed instead from the observations of Gatto<sup>41</sup> who found an increased globular resistance in both parents in all 8 families examined. The divergence from the Mendelian hypothesis suggested by Gatto was added to the fact that a first summary processing of the data showed that in families where both parents were microcythemic, 1/3 of the children had the disease, 1/3 were healthy microcythemics and 1/3 were normal, rather than 1/4, 1/2 and 1/4, respectively, as would be the case for a dominant Mendelian character; and in families with one microcythemic parent and one normal one there were 2/3 microcythemics and 1/3 normal, instead of 1/2 normal and 1/2 microcythemic.

The divergence between the expected hereditary behaviour and that actually observed was to be ascribed to diagnostic errors made in the hospitals from which the two researchers had begun their search for subjects to study and that had led to the inclusion among the case histories of subjects not affected by Cooley’s disease.

In 1946, Silvestroni and Bianco undertook a series of extensive hematologic screening campaigns in the province of Ferrara and the Rovigo area, i.e. in geographic areas with one of the highest incidences of Mediterranean anemia in Italy. Marino Ortolani, who directed the Provincial Institute for Childhood in Ferrara, had for some time been alerting the scientific community to the need to carry out investigations on Cooley’s disease in those areas in view of the high death rate recorded there.

The results of the first Ferrara campaign were presented on 30 November 1946 to the Rome Medical Academy<sup>42</sup> and later published by *Minerva Medica* in an issue however that, owing to the postwar difficulties, came out only in 1948<sup>43</sup>. The two researchers examined 1790 subjects in the Ferrara area and 603 subjects in surrounding zone in the provinces of Rovigo, Ravenna and Bologna. This time the case histories were selected with a much higher degree of precision than in the previous research. The diagnostic skills of Marino Ortolani and of the physicians in this zone had been refined by years of laborious familiarity with Cooley’s disease.

In 38 families out of 40 Silvestroni and Bianco detected the presence of microcythemia in both parents of the sick subjects and in 2 families in only one. With the exception of the latter anomaly, which only later would be accounted for in terms of molecular biology, the two researchers had succeeded in demonstrating the presence of microcythemia in both parents of the sick children on the basis of a much larger number of case histories than had ever been seen before. Furthermore, when commenting on these results, Silvestroni and Bianco emphasized how this knowledge and the methods used to detect microcythemia developed by them<sup>44</sup> finally allowed the onset of Cooley’s disease to be prevented by means of pre-marital screening of carriers<sup>45</sup>.

### *Application of Weinberg’s correction measure and the demonstration of the heterozygote/homozygote hypothesis*

However, the figure theoretically expected for children carriers was still significantly distant from the calculated value. As had already occurred in the case of studies on thalassemia genetics carried out at Rochester by William N. Valentine and James V. Neel<sup>46</sup>, an excess of microcythemics continued to be found in real cases. As for the two US authors, the excess found for children carriers depended on the fact that the test subject, i.e. the Cooley disease patient used to identify the family having microcythemics, was included in the calculation and was obviously also a child carrier. Following the example of Neel and Valentine<sup>47</sup>, Silvestroni and Bianco used case histories that had been enlarged even further (346 microcythemic families in 44 of which both parents were microcythemic) applying Weinberg’s correction method in which the test subject was excluded from the total number of children and, like the two American, they finally obtained

results that perfectly matched those expected on the basis of the heterozygote-homozygote hypothesis and Mendel's law of dominance<sup>48</sup>.

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<sup>1</sup> At the time simply called thalassaemia, or Cooley's disease or Mediterranean anemia.

<sup>2</sup> WEATHERALL D.J. *Toward an understanding of the molecular biology of some common inherited anemias: the story of thalassaemia*. In: *Blood, Pure and Eloquent*, M.M. WINTROBE (ed), McGraw-Hill, New York, 373-414, 1980. WEATHERALL D.J. *Thalassaemia: the long road from bedside to genome*. Nature Reviews Genetics, 2004, 5: 1- 7. WEATHERALL D.J., CLEGG JB *The thalassaemia syndromes*, Oxford, Blackwell Science, 2001 (see the long chapter 1 "Historical perspectives: the many and diverse routes to our current understanding of the thalassaemias, pp. 3-62).

<sup>3</sup> WINTROBE M.M. (ed) *Blood, Pure and Eloquent*, McGraw-Hill, New York, 1980. WINTROBE M.M. *Haematology – the Blossoming of Science*, Lea & Febiger, Philadelphia, 1985.

<sup>4</sup> AURICCHIO L. *Su alcune sindromi di anemia con splenomegalia a carattere familiare nell'infanzia*. La Pediatria, 1928, 36, 19: 1023-1040, p. 1035)

<sup>5</sup> *Ib.*, p. 1038

<sup>6</sup> CERZA L. *Contributo alla conoscenza delle anemie con splenomegalia a carattere familiare nell'infanzia*. La Pediatria, 1932; 40: 685-701.

<sup>7</sup> *Ib.*, p. 698

<sup>8</sup> *Ib.*, pp. 699-700.

<sup>9</sup> *Ib.*, pp. 700-701.

<sup>10</sup> ORTOLANI M. *L'anemia di Cooley in gemelle monocorie*. Il Lattante, 1936; 7: 323-353.

<sup>11</sup> CAREDDU G. *Osservazioni sulla anemia di Cooley in gemelli*. Studi Sassaresi. Sezione II – Scienze Mediche e Naturali, 1940; 18: 3-8.

<sup>12</sup> *Ib.*, p. 8.

<sup>13</sup> CROSETTI L., BAJARDI G. *Presentazione di un caso familiare di splenomegalia emolitica con note ematologiche inconsuete*. Atti della Regia Accademia dei Fisiocritici of Siena, 1935, Communication presented at the meeting of 14 June 1935. DONDI G. *Sindromi emolitiche nell'infanzia*. Ospedale Maggiore of Novara, 1936; 13, 9-10-11-12: 413-452; 460-516; 520-568. TESTOLIN M. *Splenomegalia emolitica costituzionale con aumento della resistenza globulare massima, con ovalocitosi e poichilocitosi*. Clinica Medica Italiana, 1936; 67:155-167. PONTONI L. *Aspects cliniques et pathogéniques de l'érythémie chronique familiale, type Cooley*. Revue Belgique des Sciences Médicales, 1938; 10: 216. FRANCAVIGLIA A. *Ricerche sul Cooley's disease. I – Studio su alcuni casi di Cooley's disease osservati in Puglia*. Archivio per le scienze mediche, 1939; 68, 4: 395-408. CAREDDU G. *Osservazioni sulla anemia di Cooley in gemelli*. Studi Sassaresi. Sezione II – Scienze Mediche e Naturali, 1940; 18: 3-8. COLARIZI A., BIDDAU I. *Anemie eritroblastiche splenomegaliche dell'infanzia*, Tipografia del Libro di Bortolo Bianchi, Pavia, 1940. GENTILI A. *Contributo allo studio della malattia di Cooley*. Archivio Italiano di Pediatria e Puericoltura 1940; 7: 346-389. VALLISNERI E. *Su trenta casi di sindrome anemica di Cooley*. Policlinico infantile, 1940; 4: 145-147. BRIGANTI A. *Sulla malattia di Cooley*. Haematologica, 1940; 22: 325. GATTO I *Ricerche sui familiari dei bambini affetti da malattia di Cooley. Contributo alla comprensione della ereditarietà del male*. Sezione Siciliana della Società Italiana di Pediatria, 1941; 12, 12. GATTO I. *Ricerche sui familiari dei bambini affetti da malattia di Cooley. Contributo alla comprensione della ereditarietà del male*. Rivista di Clinica Pediatrica, 1941; 14, 5: 225-252. SPECIE R. *Considerazioni a proposito di un caso di ittero emolitico con resistenza globulare aumentata*. Minerva Medica, 1941; 1: 504-508. CHITI G. *Splenomegalia emolitica costituzionale con eritrocitosi ipocromica ed aumento della resistenza globulare*. Rassegna di Fisiopatologia Clinica e Terapeutica, 1941; 13: 335. COCCHI C. *L'anemia mediterranea di Cooley. Nuovi casi osservati in Sardegna: tentativi di terapia. Ipotesi etiologiche e patogenetiche*. Rivista di Clinica Pediatrica, 1941; 39, 5: 257-287. PARADISO F., and ERNANDEZ G. *Ipoevoluitismo somatico-morfologico in bambini affetti d'anemia di Cooley*. Bollettino e Atti della Società Medico Chirurgica di Catania, 1941; 9: 15-28. FERRANNINI L. *Emo-osteo-patia di Cooley in adulto*. Minerva Medica 1942; 33, 30: 57-68.

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- <sup>18</sup> *Ib.*, p. 332.
- <sup>19</sup> *Ib.*, p. 332.
- <sup>20</sup> *Ib.*, p. 332.
- <sup>21</sup> FRONTALI G., RASI F. *L'eritroblastosi e l'emolisi nella malattia di Cooley e di Di Guglielmo*. Archivio Italiano di Pediatria e Puericoltura, 1939; 7: 259-345.
- <sup>22</sup> *Ib.*, p. 271.
- <sup>23</sup> GATTO I. *Osservazioni su ventiquattro casi di anemia eritroblastica di Cooley*. In *Atti del XVII Congresso italiano di Pediatria*, Napoli, 20-25 May 1940, parte II, Arti Grafiche Vasesiane G.B. Capelli, Varallo Sesia pp. 478-482.
- <sup>24</sup> GATTO I., note 13. GATTO I. *Ricerche sui familiari dei bambini affetti da malattia di Cooley*. Archivio Italiano di Pediatria e Puericoltura, 1942; 9: 128-168.
- <sup>25</sup> GATTO I. *Ricerche sui familiari dei bambini affetti da malattia di Cooley*. Archivio Italiano di Pediatria e Puericoltura, 1942; 9: 128-168.
- <sup>26</sup> *Ib.*
- <sup>27</sup> SILVESTRONI E. and BIANCO I. *Studio della resistenza osmotica delle emazie secondo il metodo di Simmel. Primi risultati sulla determinazione dei valori normali e ricerche sulla possibilità di utilizzare l'esame della resistenza globulare per la diagnosi di cancro*. Policlinico Sez. Med., 1943; 50: 47.
- <sup>28</sup> SILVESTRONI E. and BIANCO I. *Prime osservazioni di resistenze globulari aumentate in soggetti sani e rapporto fra questi soggetti e i malati di cosiddetto ittero emolitico con resistenze globulari aumentate*. Bollettino e Atti dell'Accademia Medica di Roma, 1943; 69, 11-12: 293-309, sitting of 26 November 1943.
- <sup>29</sup> *Ib.*, p. 304.
- <sup>30</sup> *Ib.*, p. 304.
- <sup>31</sup> The thalassaemic trait in current nomenclature.
- <sup>32</sup> SILVESTRONI E. and BIANCO I. *Sull'esistenza nell'uomo di una particolare anomalia ematologica costituzionale*. Bulletin and Proceedings of Accademia Medica of Rome, 1944-45; 70: 44-49, sitting of 24 December 1944. Published in unabridged form: SILVESTRONI E and BIANCO I *Dimostrazione nell'uomo di una particolare anomalia ematologica costituzionale e rapporti fra questa anomalia e l'anemia microcitica costituzionale*. Policlinico Sez. Med., 1945; 52: 105-137.
- <sup>33</sup> SILVESTRONI E. and BIANCO I. *Ricerche sui familiari sani di malati di Cooley's disease*, Ricerche di Morfologia, 1946; 22: 217-256.
- <sup>34</sup> SILVESTRONI E. and BIANCO I. *Microcitemia e morbo di Cooley*. Bollettino e Atti dell'Accademia Medica di Roma, 1945-46 and 1946-47; 71: 3-4, sitting of 29 December 1945.
- <sup>35</sup> SILVESTRONI E. and BIANCO I., notes 34 and 35.
- <sup>36</sup> *Ib.*
- <sup>37</sup> SILVESTRONI E. and BIANCO I. *Microcitemia*. Bollettino e Atti dell'Accademia Medica di Roma, 1945-46 e 1946-47; 71: 7, sitting of 13 April 1946, p. 7.
- <sup>38</sup> *Ib.*, p. 7.
- <sup>39</sup> SILVESTRONI E. and BIANCO I. *Ricerche sui familiari sani di malati di Cooley's disease*, Ricerche di Morfologia, 1946 22: 217-256. SILVESTRONI E. and BIANCO I. *Una particolare anomalia ematologica: la "microcitemia"*. Minerva Medica, 1946; 37: 206-221.
- <sup>40</sup> CAMINOPETROS J. *L'anémie érythroblastique des peuples de la méditerranée orientale*, Monographies de l'Académie d'Athènes, 1937 ; 6, 3: 81-143, plus 59 figures.
- <sup>41</sup> GATTO I., note 26.
- <sup>42</sup> SILVESTRONI E. and BIANCO I. *Nuove ricerche sui famigliari di malati di morbo di Cooley e prime osservazioni sulla frequenza dei portatori di microcythemia nel ferrarese e in alcune regioni limitrofe*. Bollettino e Atti dell'Accademia Medica di Roma, 1945-46 e 1946-4; 72: 32-33 sitting of 30 November 1946.
- <sup>43</sup> SILVESTRONI E. and BIANCO I. *Nuove ricerche sull'eziologia del Cooley's disease e prime osservazioni sulla frequenza della microcitemia nel Ferrarese*. Minerva Medica, 1948; 39, 1, 8: 8-21.
- <sup>44</sup> SILVESTRONI E. and BIANCO I. *Il metodo di Simmel per lo studio delle resistenze globulari*. Policlinico Sez. Prat., 1945; 51: 153-158.
- <sup>45</sup> SILVESTRONI E. and BIANCO I. *Nuove ricerche sui famigliari di malati di Cooley's disease e prime osservazioni sulla frequenza dei portatori di microcythemia nel ferrarese e in alcune regioni limitrofe*. Bollettino e Atti dell'Accademia Medica di Roma, 1945-46 e 1946-47; 72: 32-33 sitting of 30 November 1946.
- <sup>46</sup> VALENTINE W.N., NEEL J.V. *Hematologic and genetic study of the transmission of thalassemia (Cooley's anemia; Mediterranean anemia)*. Archives of internal medicine, 1944; 74: 185-196. NEEL J.V., VALENTINE W.N. *Further studies on the genetics of thalassemia*. Genetics, 1947; 32: 38-63.
- <sup>47</sup> VALENTINE W.N., NEEL J.V., note 47.
- <sup>48</sup> SILVESTRONI E. and BIANCO I. *Sulla frequenza dei portatori di microcythemia nel Ferrarese, sui gruppi sanguigni dei microcitemici e sulla trasmissione ereditaria della microcythemia*. Ricerca Scientifica e Ricostruzione, 1947; 17, 12: 2021-2027. SILVESTRONI E. and BIANCO I. *Nuove ricerche sulla trasmissione ereditaria della microcythemia*. Policlinico, Sez. Prat., 1947; 54, 50: 1359- 1370.