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**A Lucky Mistake:
The Splenic Glands of Marcello Malpighi**

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Short title: The Spleen in Marcello Malpighi's work

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Abstract

Marcello Malpighi (1628-1694) is one of the most important anatomists and physicians in the History of Medicine. His contributions to the understanding of human anatomy and physiology span from the first description of capillary circulation to a thorough analysis of the structure and function of body glands. Malpighi believed that most organs consisted of glandular structures, whose distribution and microscopic features determine each organ specific function. He also applied this view to the study of spleen anatomy, which he recognized as composed of two distinct anatomic compartments (i.e. the red and the white pulp). Malpighi's observations on the structure and function of the spleen were first published in 1666 in *De Viscerum Structura*. In this paper, we pay tribute to this work, presenting Malpighi's theory of the spleen as a glandular organ. The rationale of Malpighi's view and its value for contemporary pathologists and medical researchers will also be elucidated.

1. Introduction

Marcello Malpighi (1628-1694) is one of the greatest physicians and biologists of the Seventeenth century (Figure 1). Born into a wealthy family from central Italy, Malpighi studied Medicine at the University of Bologna. Here, he became a professor of Medicine and devoted himself to the study of microscopic anatomy. This led him to pivotal achievements in the field of anatomy and histology, including the discovery of capillary vessels and pulmonary alveoli and the first description of squamous epithelia and blood cells. These achievements gained him worldwide recognition. He became the personal physician of pope Innocent XII and was the first Italian to be admitted as an honorary member to the Royal Society [1].

One of Malpighi's most important works is *De Viscerum Structura*, a treatise in four essays, discussing the microscopic anatomy of the liver (*De Hepate*), the kidney (*De Renibus*), the cerebral cortex (*De Cortice Cerebri*) and the spleen (*De Liene*) [2]. This work wiped out long-lasting dogmas on human anatomy and physiology, laying the foundations for new, scientifically-based medical knowledge.

Malpighi believed that most tissues consisted of elementary anatomic modules, which he recognized as "glands". The different distribution and microscopic features of these glandular units determined each organ specific function. In Malpighi's view, glands consisted of minute cavities, equipped with excretory ducts, surrounded by blood vessels and nerves (Figure 2A). Their function was essentially to filter blood and to produce different types of secretions [2,3].

Malpighi believed that a huge variety of organs were made up of glands. In *De Viscerum Structura*, he reported experimental evidence that glands are indeed present in the kidney, the liver, the brain and the spleen. While Malpighi's achievements on renal and hepatic anatomy are widely acknowledged, legitimate questions remain in regards to the "glands" he observed in the cerebral cortex and the spleen. Recent studies have

demonstrated that Malpighi's cerebral glands are artifacts due to inadequate fixation and processing [4]. The history of splenic glands, however, is more complex and intriguing.

In this paper, we pay tribute to Marcello Malpighi's view of spleen anatomy and physiology. To this aim, we will examine selected passages from *De Liene*, to investigate the logical and scientific roots that supported Malpighi's theories on splenic structure and function.

2. Malpighi and the Red Pulp

One opinion seems to be shared by all Anatomists: that the spleen originates from some kind of clotted blood, which anchors and fixes vessels that arise from it. As such, the spleen would have some features in common with other organs such as the heart, the liver and the kidneys. Long and laborious research, however, did give me the opportunity to better understand the structure of the spleen, which now seems more clear and precise. The splenic parenchyma is in fact a patchwork of membranes surrounding small chambers and cells.

Malpighi is rarely remembered as the first anatomist who described the red pulp. In fact, he devoted many efforts to elucidate the intricate network of channels and cavities, which constitute the majority of the splenic tissue. He succeeded by applying macro- and microscopic analyses, which helped him identify fibrous septa originating from the splenic capsule and branching into the red pulp. These fibrous strands ultimately circumscribe blood-containing spaces, referred to as "chambers" or "cells" (Figure 2B). Such a thorough description constitutes the first modern account of red pulp sinuses and cords. Malpighi's achievement is noteworthy as it challenges long-standing theories, which had considered the spleen to be a mere clot of blood [5].

The method he used to clarify such an anatomic structure deserves special consideration, as it provides invaluable insight into the seventeenth-century approach to medical and biological issues.

To make this structure obvious to all, shall the splenic artery be isolated and knotted up; aftermath, shall air be blown through the splenic vein with a syringe or directly by mouth. The whole spleen will immediately become turgid and of increased volume [...]. If, on the other hand, one dries out and cuts such a swollen spleen, he will notice huge numbers of small chambers and cells, similar to a honeycomb.

For his research activities Malpighi adopted revolutionary methods and new experimental settings. As a former student of the physicist Giovanni A. Borelli (1608-1679), he systematically applied the scientific method to elucidate the nature of tissues and organs. Spleen inflation and exsiccation are paradigmatic examples of such an approach, as they are specifically aimed to unveil the fine structure of the red pulp: air inflation contributes to the expansion of splenic sinuses to a degree that makes them appreciable even on gross inspection; exsiccation contributes to “fixation” of this anatomic artifact, facilitating spleen sectioning and parenchymal examination. Thanks to Malpighi, this procedure was largely credited throughout Europe and widely applied by several authors, including Hermann Boheraave (1668-1738), Philip Verheyen (1648-1710), and Leopoldo Marco Antonio Caldani (1723-1813) [6-8].

3. Malpighi and the White Pulp

We have already noticed the extraordinary ingenuity of Nature in organizing the structure of the spleen. In general, it adopts one way to operate and this, as said, is simple: for this reason, Nature has added some parts to the spleen, similar to those observed in other organs. In the spleen, therefore, there are nodular aggregates of glands (or vesicles or sacs), dispersed throughout the parenchyma and resembling bunches of grapes.

Malpighi's description of the white pulp represents his greatest contribution to the study of spleen anatomy. In the fifth Chapter of *De Liene* (titled: *On some corpuscles dispersed throughout the Spleen*), he reports the results of thorough anatomic studies by demonstrating the existence of small, grape-like nodular bodies in the spleen of most

animals. To interpret their nature, Malpighi applies a principle that inspired his whole view of biology, whereby Nature operates by similar rules in different settings. Moving from this assumption, Malpighi observes that splenic corpuscles are juxtaposed to small intra-parenchymal arteries, surrounding them like “tendrils or climbing ivy”. As these structures are often observed in glandular organs (e.g. the kidney or the liver), Malpighi concludes that splenic corpuscles are microscopic glands (Figure 1B). He nonetheless recognizes that such glandular units have unique morphologic/functional features, including: (i) a vesicle/sack-like shape; (ii) the lack of excretory ducts; and (iii) the lack of an overt secretory activity.

For a modern reader, Malpighi's glands clearly represent nodular lymphoid aggregates of the white pulp (i.e. splenic lymphoid follicles). In the seventeenth century, however, the distinction between properly-defined glands and lymphoid organs was blurred, as the term “gland” was broadly applied to any organ resembling an “acorn cluster” (the Latin word for “gland” [*glandula*] indeed signifies “tiny acorn”). Such “glands” were sub-classified into conglomerate and conglobate: the former had jagged, irregular contours and correspond to the current exocrine/endocrine glands; the latter had smoother and regular shape and include the lymph nodes and lymphoid organs [9-10].

Despite the fact that the nature and composition of the lymphoid system would be unveiled over three centuries later [10], a passage of *De Liene* may suggest Malpighi's partial understanding of the white pulp function. He observes:

Splenic glands are not easily identifiable in all animals: in the ox, goat and sheep they are demonstrable even by coarse sectioning and become more evident after scratching the cut surface with a knife, or washing it under water. In humans, they are instead more difficult to detect, but become evident and bulky in diseases that enlarge all body glands.

By comparative anatomic studies, Malpighi reports that “splenic glands” are smaller and less evident in humans than in other animals. He also notices that the

size of human glands vary depending on specific disease conditions. This observation prompts him to consider splenic glands as part of a widespread “glandular” apparatus, corresponding (as we now know) to the lymphoid system. The gland-enlarging processes, which Malpighi refers to, may indeed represent infectious, inflammatory or lymphoproliferative disorders, causing systemic lymphadenopathy and white pulp expansion. Based on these observations, Malpighi's *De Liene* possibly contains one of the very first (yet still implicit) descriptions of the immune system.

4. Malpighi and the spleen function

What one should then think on the function of such a complex organ, I totally ignore. For this reason, I will only receive as probable what I will be able to infer from its anatomical structure, despite I am aware that these are still preliminary opinions, which will be abandoned as soon as better ones are advanced. Since, then, the spleen is composed of countless oval corpuscles or glands, it will probably operate some blood separation.

In the last part of the essay, Malpighi moves from mere anatomic to functional considerations. In particular, he applies syllogistic reasoning to his anatomic discoveries, thereby posing major and minor premises to get to novel conclusions on spleen physiology. Malpighi's argument may be summarized as follows: (i) as all glands perform some form of blood filtration (*major premise*) and (ii) the spleen consists of innumerable glands (*minor premise*), then (iii) the spleen performs some form of blood filtration (*conclusion*).

This conclusion highlights a remarkable (yet still incomplete) understanding of spleen physiology. Malpighi's hypothesis concerning blood filtration indeed sums up the recognized functions of the red pulp, which include: (i) culling senescent and/or damaged red blood cells (RBCs); (ii) pitting RBCs inclusions and/or intra-cellular parasites; and (iii) maintaining iron homeostasis by recycling heme groups [11]. Malpighi's theory also fits

with the pathophysiology of several hematological disorders, which lead to red pulp entrapment of blood cells (e.g. sickle cell disease, hemoglobinopathies, and extra-medullary hematopoiesis in Philadelphia-negative myeloproliferative neoplasms) [12-13].

By contrast, Malpighi's view of the spleen as a "secreting" (i.e. "glandular") organ is partially in accordance with the white pulp function. This anatomic compartment is indeed composed of cuffs of lymphocytes and plasma cells. The latter produce large amounts of soluble immunoglobulins, which are secreted in the splenic sinusoids to reach the bloodstream. Far from being a true "glandular" activity, this "secretory" immunologic function is, however, similar to the one originally postulated in *De Liene*.

Quite understandably, Malpighi was unaware of immunoglobulins, plasma cells and acquired immunity. In order to characterize the nature and function of the splenic filtrate, he thus moved from simple anatomic observations, sustaining that this humor favors bile production in the liver:

If, as we believe, the main function of the liver is to produce bile, this activity seems to be favored by the splenic juice, which is intermixed with the blood reaching the liver. In particular, the splenic humor (thanks to its driving force and to its ability of solving stable bonds) favors the separation of those parts, which are intended to become bile.

To sustain such hypothesis, Malpighi performs an *in vivo* experiment by ligating the splenic vessels of a dog (thus inducing functional splenectomy). Much to his surprise, however, he does not observe any anatomic and/or functional impairment, as the dog rapidly recovers from the operation and demonstrates good appetite and temperament. The only consequence of surgery is the late onset of a right hypochondrium mass, which turns out to be massive hepatomegaly at *post mortem* examination. The reasons for such a liver enlargement are not investigated further by Malpighi, as he hardly relates them to the ligation of the splenic vessels.

While this experiment adds little to the understanding of spleen physiology, it helps Malpighi refute several long-standing and erroneous beliefs on the splenic function. In particular, Malpighi criticizes Galen's and Plato's opinion that the spleen purifies the hepatic blood from earthy and melancholic humors [5,14]. He indeed observes that: (i) the blood only flows from the spleen toward the liver (and not the opposite way); and (ii) the blood composition of the splenic artery does not significantly differ from that of other anatomic districts. He also rejects Galen's opinion, by which the spleen would induce hunger by secreting peculiar spirits into the gastric cavity [5,14]. In fact, he notices that: (i) his dog did have excellent appetite even after functional splenectomy; and (ii) the veins between the spleen and the stomach have valves that impede the upward movement of any fluid or spirit. The latter observation also leads him to refuse Aristotle's idea that the spleen secretes acid humors into the stomach to facilitate food digestion [5]. Malpighi finally rejects Bartholin's view of the spleen as a "left-sided liver" producing blood to nourish the abdominal organs [14]. In fact, the abdominal viscera of the splenectomized dog did not show any change at autopsy examination.

Despite the lack of any evidence that the spleen favored bile production, Malpighi's theory received major credit by several Authors. His idea was embraced by the great Dutch physician, Herman Boerhaave, and by the founder of modern Pathology, Giovanni Battista Morgagni (1682-1771) [6,15]. Similar opinions were shared by other anatomists, such as Philip Verheyen [7] and Leopoldo Marco Antonio Caldani [8]. It was only in the twentieth century, with the discovery of the lymphoid system and the characterization of the immune reactions that the limits of Malpighi's hypothesis were definitively demonstrated.

5. Conclusions

The history of mistakes is more informative than that of great successes. Mistakes are indeed invaluable tools to understand the scientists' logical approach to complex and unprecedented issues.

Malpighi's *De Liene* is a perfect example of such a principle. Dealing with a largely neglected and still poorly understood organ, Malpighi was forced to apply a rigorous method to his investigation. This led him to great anatomic achievements (white pulp follicles would henceforth be termed "Malpighian corpuscles") and to an integrated view of human physiology. Of note, it also prompted erroneous conclusions on spleen physiology and function, which largely stemmed from Seventeenth-century scientific and epistemological issues. These inconsistencies are of paramount importance to understand the cultural *milieu*, in which scientists and physicians of this period were operating.

In conclusion, Malpighi's *De Liene* represents a milestone in the History of Medicine for its fundamental contributions to the understanding of spleen anatomy and function. The epistemology of Malpighi's research also constitutes an invaluable legacy for any scientist involved in biomedical research activities.

Figure legend

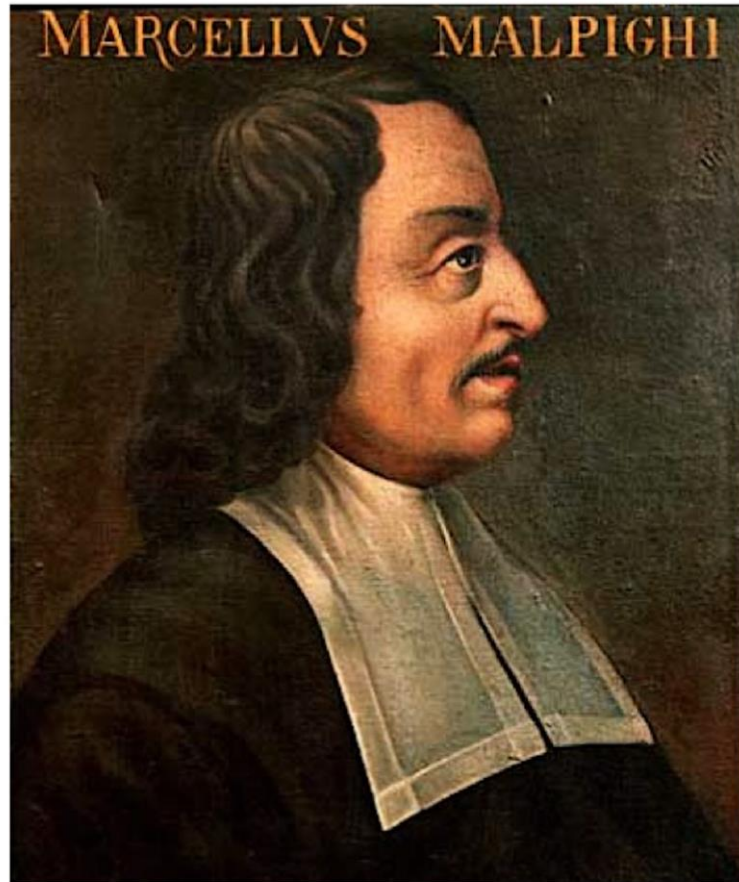
Figure 1. Marcello Malpighi (1628-1694). This eighteenth-century portrait of unknown Author is currently located in the Hall of Medicine at Bo Palace – University of Padova (Padova, Italy). It was likely part of a private collection belonging to Giovanni Battista Morgagni, the founder of Anatomic Pathology and former Professor at this University.

Figure 2. The microscopic anatomy of glands according to Marcello Malpighi.

A. In Malpighi's view, glands consist of small empty cavities provided with afferent and efferent vessels, which carry the blood to filter. The product of such filtration is then secreted into specific ducts, residing opposite to the vascular pole. **B.** Splenic glands basically recapitulate these general features, even though they show some tiny (yet crucial) differences: (i) splenic glands are not provided with a true excretory duct, as they secrete their filtrate directly into the splenic vein branches; (ii) afferent and efferent vessels reside at opposite glandular poles; (iii) each glandular unit lays within a small chamber (or "cell") whose walls are constituted by the ramifications of intra-parenchymal fibrous septa.

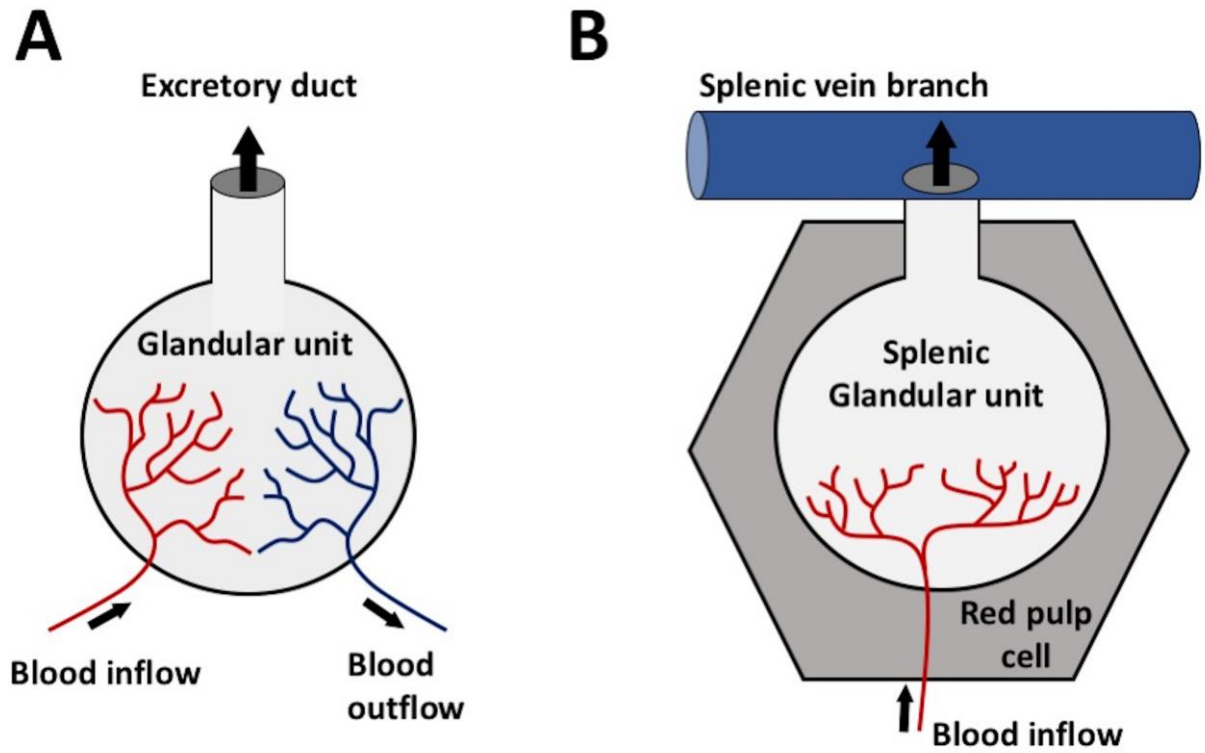
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Pizzi *et al* – Figure 1

16.

Pizzi *et al* – Figure 2

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