






Brief Report

Autonomic and Vascular Responses during Reactive Hyperemia in Healthy Individuals and Patients with Sickle Cell Anemia

Erislandis López-Galán ¹, Adrián Alejandro Vitón-Castillo ², Ramón Carrazana-Escalona ³, Maylet Planas-Rodríguez ¹, Adolfo Arsenio Fernández-García ⁴, Ileana Cutiño-Clavel ¹, Alexander Pascau-Simon ⁵, Philippe Connes ⁶, Miguel Enrique Sánchez-Hechavarría ^{7,8} and Gustavo Alejandro Muñoz-Bustos ^{9,*}

¹ Departamento de Ciencias Básicas Biomédicas, Facultad de Medicina, Universidad de Ciencias Médicas de Santiago de Cuba, Santiago de Cuba 90100, Cuba; maylet.planas@infomed.sld.cu (M.P.-R.); ileana.clavel@infomed.sld.cu (I.C.-C.)

² Facultad de Ciencias Médicas “Dr. Ernesto Che Guevara de la Serna”, Universidad de Ciencias Médicas de Pinar del Río, Pinar del Río 20100, Cuba; adrianviton964@gmail.com

³ Departamento de Ciencias Clínicas Básicas, Facultad de Medicina, Universidad Católica de la Santísima Concepción, Concepción 4090541, Chile

⁴ Facultad de Ciencias Naturales y Exactas, Universidad de Oriente, Santiago de Cuba 90100, Cuba; adolfof@uo.edu.cu

⁵ Hospital General “Dr. Juan Bruno Zayas Alfonso”, Laboratorio Vascular no Invasivo, Santiago de Cuba 90400, Cuba; alexander.pascau@gmail.com

⁶ LIBM Laboratory, Team “Vascular Biology and Red Blood Cell”, Claude Bernard University Lyon 1, 69622 Lyon, France

⁷ Grupo Bio-Bio Complejidad, Departamento de Ciencias Clínicas y Preclínicas, Facultad de Medicina, Universidad Católica de la Santísima Concepción, Concepción 4090541, Chile

⁸ Núcleo Científico de Ciencias de la Salud, Facultad de Ciencias de la Salud, Universidad Adventista de Chile, Chillán 3780000, Chile

⁹ Facultad de Ciencias de la Salud, Sede Concepción, Universidad de las Américas, Concepción 4030000, Chile

* Correspondence: g.munoz.bustos@gmail.com



Citation: López-Galán, E.; Vitón-Castillo, A.A.;

Carrazana-Escalona, R.;

Planas-Rodríguez, M.;

Fernández-García, A.A.;

Cutiño-Clavel, I.; Pascau-Simon, A.;

Connes, P.; Sánchez-Hechavarría,

M.E.; Muñoz-Bustos, G.A.

Autonomic and Vascular Responses

during Reactive Hyperemia in

Healthy Individuals and Patients

with Sickle Cell Anemia. *Medicina*

2023, 59, 1141. <https://doi.org/10.3390/medicina59061141>

Academic Editor: Thomas Kühne

Received: 12 February 2023

Revised: 7 April 2023

Accepted: 10 May 2023

Published: 13 June 2023



Copyright: © 2023 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article

distributed under the terms and

conditions of the Creative Commons

Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: *Background and Objectives:* To compare autonomic and vascular responses during reactive hyperemia (RH) between healthy individuals and patients with sickle cell anemia (SCA). *Materials and Methods:* Eighteen healthy subjects and 24 SCA patients were subjected to arterial occlusion for 3 min at the lower right limb level. The pulse rate variability (PRV) and pulse wave amplitude were measured through photoplethysmography using the Angiodin[®] PD 3000 device, which was placed on the first finger of the lower right limb 2 min before (Basal) and 2 min after the occlusion. Pulse peak intervals were analyzed using time–frequency (wavelet transform) methods for high-frequency (HF: 0.15–0.4) and low-frequency (LF: 0.04–0.15) bands, and the LF/HF ratio was calculated. *Results:* The pulse wave amplitude was higher in healthy subjects compared to SCA patients, at both baseline and post-occlusion ($p < 0.05$). Time–frequency analysis showed that the LF/HF peak in response to the post-occlusion RH test was reached earlier in healthy subjects compared to SCA patients. *Conclusions:* Vasodilatory function, as measured by PPG, was lower in SCA patients compared to healthy subjects. Moreover, a cardiovascular autonomic imbalance was present in SCA patients with high sympathetic and low parasympathetic activity in the basal state and a poor response of the sympathetic nervous system to RH. Early cardiovascular sympathetic activation (10 s) and vasodilatory function in response to RH were impaired in SCA patients.

Keywords: sickle cell anemia; pulse rate variability; pulse wave amplitude; autonomic nervous system

1. Introduction

Sickle cell anemia (SCA) is an autosomal recessive disorder characterized by a single mutation in the β -globin gene, which leads to the production of abnormal hemoglobin (Hb) called HbS. When deoxygenated, HbS polymerizes, and a mechanical distortion of red

blood cells (RBCs) occurs, i.e., sickling [1]. Sickle RBCs are fragile and very rigid, which makes patients prone to chronic hemolytic anemia, and frequent and recurrent painful vaso-occlusive crises (VOC) [1,2]. A wide variety of acute complications may occur in SCA, reflecting the complex pathophysiology of this disease [3].

It has been established that blood rheological alterations [2], chronic vascular inflammation, abnormal adhesion processes [3,4], and vascular dysfunction [5] play an important role in the occurrence of sickle cell anemia complications. One of the characteristic complications of sickle cell anemia are the vaso-occlusive crises, where it has been suggested that interactions between sickle erythrocytes and the vascular endothelium may contribute to its pathogenesis. Sickle red blood cells can occlude microvessels directly, by adhering to the endothelium, or indirectly, by disrupting endothelial functions. Interactions between sickle red blood cells, and the vascular endothelium and abnormal regulation of vasomotor tone may synergistically contribute to the occurrence of a vaso-occlusive crisis [6].

More recently, autonomic nervous system (ANS) dysfunction has been demonstrated to be implicated in the pathophysiology of SCA [7]. A recent study suggested that autonomic activity might be reduced in participants with sickle cell anemia during vaso-occlusion [8]. Inamo et al. [9] observed a lower mean ANS activity in SCA patients at baseline compared to healthy controls, but the degree of depression of autonomic activity greatly varied among patients. It has been suggested that there may be a relationship between autonomic reactivity and the clinical severity of patients with sickle cell anemia [10,11]. However, although ANS activity could be involved in the pathophysiology of SCA, the mechanisms through which an autonomic imbalance may modulate the clinical severity of disease are still poorly understood [12–14].

Several tests are available for the evaluation of the autonomic function, such as plasma catecholamines measurement, baroreflex sensitivity tests, and muscle sympathetic nerve activity [15]. However, these tests are expensive, time-consuming, and require well-trained personnel [16]. On the other hand, cardiovascular autonomic reflex testing (CART) has been considered the gold standard for the diagnosis of cardiovascular autonomic neuropathy (CAP), although some authors found this test difficult to apply and it requires the cooperation of patients [17]. ANS activity is commonly assessed through the quantitative assessment of heart rate variability (HRV), which reflects the sympathetic and parasympathetic control of the sinoatrial node [18,19].

Many works have found that pulse rate variability (PRV), which is extracted from photoplethysmography (PPG) data, can serve as an alternative measurement of ANS activity [20–22]. PPG is a simple and low-cost optical measurement technique that can be employed to detect blood volume changes in the microvascular bed of a tissue. Furthermore, it is generally accepted that PPG can provide relevant information about the cardiovascular system, such as heart rate, arterial pressure, stiffness index, pulse transit time, pulse wave velocity, cardiac output, arterial compliance, and peripheral resistance [22].

Reactive hyperemia (RH) is the increase in blood flow, within a given vascular territory, in response to an occlusion time [23]. Several factors have been implicated in the origin of this response, such as metabolic and endothelial vasodilators, and sensory nerves. RH is a relatively simple, reliable, and non-invasive test which is commonly used to assess endothelial function [24–26], but this test can also affect ANS activity, thus expanding its clinical applications [23]. RH can be quantified as a change in perfusion pressure, as measured by plethysmography [27]. However, to our knowledge, no studies have investigated the dynamic changed in ANS during RH in SCA patients. The present research aims to compare the variations in pulse wave amplitude, and autonomic and vascular responses during reactive hyperemia (RH) between healthy subjects and SCA patients.

2. Methods

2.1. General Description

A non-observational trial was conducted with 18 healthy subjects (HbAA genotype) and 24 patients with SCA (HbSS genotype) to study the effects of RH on vascular and cardiac activity. The sickle cell anemic patients were recruited at the Special Hematology Clinic of the “Juan Bruno Zayas Alfonso” General Teaching Hospital in Santiago de Cuba, during the 2013–2014 period. Before enrollment, all patients and control subjects had clinical examination with blood pressure measurements to check for the absence of reactive hyperemia contraindication. The participants were in a resting state for the first 10 min, under controlled ambient temperature (24–27 °C) and luminance (dim light) conditions. PPG was then used to record the pulsatile volume flow at the first finger of the lower right limb, both at baseline and during a post-occlusion hyperemia test. This research was approved by the Committee of Medical Ethics of Santiago de Cuba University of Medical Sciences and conducted in the Laboratory of Basic Sciences of the same institution. All subjects gave written informed consent and were in a clinical steady-state phase of their disease, with no painful crisis and blood transfusion within the previous three months.

2.2. Exclusion Criteria

The exclusion criteria were as follows: patients receiving treatment with hydroxyurea, central or peripheral nervous system disorders (stroke or neuromuscular disorders), skin disorders, implanted electronic devices (pacemakers or implantable cardioverter defibrillators), upper or lower limb amputations, women who were pregnant or menstruating, and any type of heart arrhythmia.

2.3. Physiological Recording

The PPG technique was used to record the arterial pulse wave in the first finger of the lower right limb using the ANGIDIN[®] 18 digital plethysmograph (Biofísica Médica, Santiago de Cuba, Cuba), which captures the volume variations of the pulse wave via the reflection method (at 850 nm with a 3.5-mA photodiode). The PPG signal was then passed to an analog band pass filter from 0.1 to 15 Hz (Butterworth 5th order filter) and digitized at a resolution of 8 bits (106 samples per second). The recorded data were transmitted, processed and archived using the VAPLET[®] auxiliary software (Biofísica Médica, Santiago de Cuba, Cuba). This software allows for the exportation of the signal in a beat-by-beat manner, such that it can then be processed by the Octave software 2019 (version 5.1.0; <https://www.gnu.org/software/octave/download.html> (accessed on 5 January 2021)) (Figure 1).

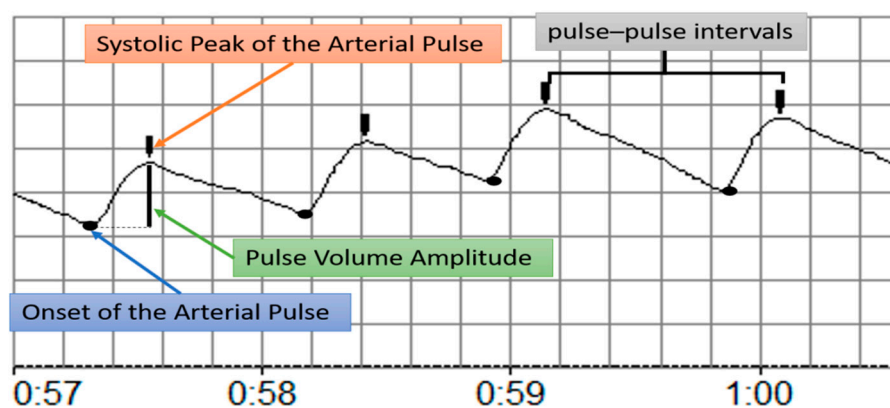


Figure 1. Schematic of physiological measurements in a photoplethysmography recording of the arterial pulse wave.

The onset and systolic peak of the arterial pulse wave were detected to calculate the pulse–pulse intervals (PPIs) and pulse volume amplitude (PVA) using the model proposed by Pascau-Simón [28]. The sphygmomanometer cuff remained fastened to the lower right limb to induce an arterial occlusion. The entire recording period comprised a 2 min baseline phase, 3 min arterial occlusion phase, and 2 min post-cuff deflation phase (RH phase). After recording the PPG signals during the 2 min baseline phase, an arterial occlusion was induced in the test limb using supra-systolic cuff pressure (50 mmHg above the subject’s baseline systolic pressure); signal measurement continued throughout the occlusion period. Occlusion was confirmed by constantly verifying the absence of a pulse waveform signal from the computer display. The cuff pressure was released completely after 3 min of arterial occlusion and the recordings continued during RH. There were no complications in the SCA patients and healthy subjects during the test performance. All measurements were obtained by the same person.

To establish the PRV, the PPIs of high-frequency (HF: 0.15–0.4) and low-frequency (LF: 0.004–0.15) bands were analyzed, as recommended by the International Consensus of Experts on HRV (1996) [29]. Time–frequency methods allow for changes to be tracked during the spectral analysis of HRV. Continuous wavelet transform (Morlet wavelet; CWT-Morlet) was used to calculate the PRV [30]. The results of the CWT-Morlet analysis were exported as a “.txt” file. A matrix of the results was generated for each subject (based on 2 min of recording) and interpolated at 2 Hz (0.5 s); in total, there were 240 PRV values.

2.4. Statistical Analysis

For the comparison of the groups, a *t*-test of independent samples was applied for the variables that presented a normal distribution and the Mann–Whitney U test for those that did not. The data are expressed as the mean \pm standard deviation (SD). Mathematical analyses and dynamic graphs (Figures 2 and 3) were performed and generated, respectively, using the Octave software 2019 (version 5.1.0; <https://www.gnu.org/software/octave/download.html> (accessed on 15 January 2021)). The average of the LF/HF matrices for the PRV was calculated from the CWT-Morlet analysis during the 2 min period after cuff deflation and interpolated at 2 Hz (0.5 s). Statistical analyses were performed with the JASP software (version 0.16; <https://jasp-stats.org> (accessed on 31 January 2021)). Significance was defined as $p < 0.05$.

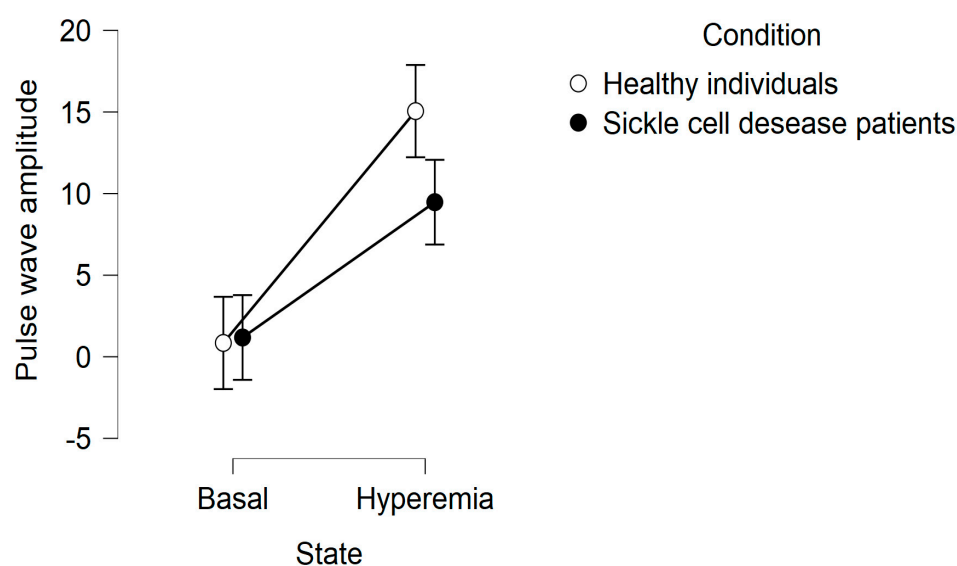


Figure 2. Pulse wave amplitude in the basal state and during the post-occlusion hyperemia test.

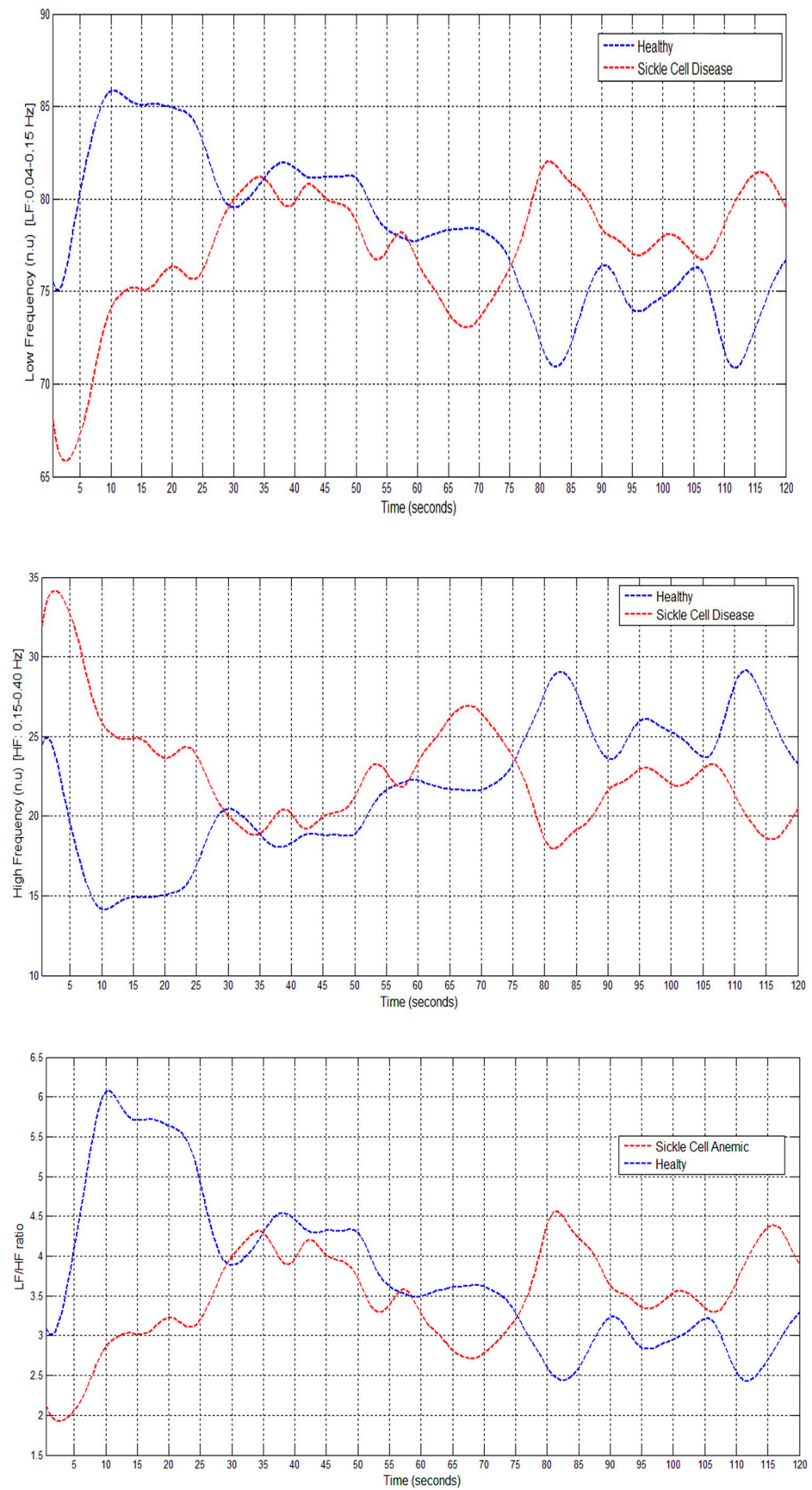


Figure 3. Dynamic autonomic response: time–frequency analysis of pulse rate variability during the post-occlusion reactive hyperemia test in healthy individuals and SCA patients.

3. Results

The patients with SCA and the control healthy subjects were similar regarding age, sex, body mass index, systolic blood pressure (SBP), and heart rate at rest. The weight, height, diastolic blood pressure, and mean blood pressure levels were significantly lower in the SCA patients than in the controls (Table 1).

Table 1. Population characteristics of the healthy controls and SCA patients.

	AA (n = 18)	SS (n = 24)	p
Sex, male/female	7/11	10/14	0.856
Weight, kg	71.9 ± 9.27	53.25 ± 14.20	<0.001
BMI	26.47 ± 3.86	24.46 ± 7.76	0.077
Age	44.5 ± 11.6	43.29 ± 11.31	0.737
Height, cm	160 ± 0.07	150 ± 0.18	0.005
SBP, mmHg	121.66 ± 6.18	114.58 ± 15.03	0.068
DBP, mmHg	81.11 ± 6.76	73.75 ± 11.34	0.019
MBP, mmHg	94.62 ± 6.06	87.36 ± 11.67	0.021
HR, beats/min	78.17 ± 4.82	75.46 ± 8.46	0.311
Hb, g/L	-	76.71 ± 18.46	-
Ht	-	0.24 ± 0.059	-

AA, healthy controls; SS, SCA patients; n, number of subjects; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean blood pressure; HR, heart rate; Hb, hemoglobin; Ht, hematocrit.

In the basal state, no statistically significant differences were found in the amplitude of the pulse wave between the healthy subjects and patients with sickle cell anemia. However, after reactive hyperemia, the post-occlusion pulse wave amplitudes were higher in healthy subjects compared to SCA patients (Figure 2). An increase in the LF/HF ratio was observed in healthy individuals at 10 s post-occlusion (early cardiovascular sympathetic activation). However, this was not observed in the SCA patients; instead, the LF/HF ratio increased 35 s post-occlusion and was higher than in healthy patients 75 s after cuff release (late cardiovascular sympathetic activation). The parasympathetic activity measured by HF was greater in SCA patients than in healthy controls after RH (Figure 3).

4. Discussion

In the present study, an impaired sympathetic activity in response to RH was observed in SCA patients. Previous studies have demonstrated that ANS activity is impaired in SCA patients compared to healthy individuals [7,31,32]. Nebor et al. [14] found lower parasympathetic activity and a greater sympatho-vagal imbalance in SCA patients with frequent pain crises than SCA patients without frequent pain crises and the control group. Charlot et al. [8] showed a higher sympatho-vagal imbalance in SCA patients during vaso-occlusive crises compared to steady-state, and that the length of hospitalization was related to the magnitude of the increase in ANS activity during the crisis.

Diastolic blood pressure (DBP) and mean blood pressure (MBP) levels were significantly lower in SCA patients than healthy control groups. These findings differ from what has been reported by other authors who have not observed such differences [7,32]. However, SCA patients had a higher SBP, DBP, and heart rate during vaso-occlusive crises (VOC) compared to steady state [8]. In addition, a greater vasoconstrictor response of neural origin has been demonstrated in SCA patients compared to healthy subjects, and that may play a role in the transition from steady-state to VOC [31]. In the present study, the patients were in a steady state, but with a sympatho-vagal imbalance, which may partially explain the lower levels of DBP and MBP.

The dynamic changes in ANS that occur during the activation maneuvers can be determined through the time–frequency methods used in the present study. The pattern of ANS activation during reactive hyperemia differs between SCA patients compared to healthy subjects. A quick sympathetic nervous system response, reflected in increments in the LF and the LF/HF ratio, was observed in healthy subjects after occlusion in this study. This has been interpreted as an early cardiovascular sympathetic reflex (10 s after occlusion) in response to RH caused by the metaboreflex [33]. The accumulation of metabolites during exercise has been shown to activate the metaboreflex [34,35]. In our SCA patients, although sympathetic activity in response to RH was impaired, it was greater immediately after the RH maneuver compared to the healthy controls, reflecting an imbalance in the ANS activity in these patients. Thus, time–frequency methods allow for the measurement of changes that might not be detected by classical methods.

In addition, the parasympathetic activity (i.e., HF) was greater in SCA patients than in healthy controls after RH. This indicates an exaggerated parasympathetic response that modifies reflexive sympathetic activity arising in response to RH in SCA patients. However, parasympathetic activity immediately after RH was lower in SCA patients than in healthy subjects. The low baseline levels of parasympathetic activity in these subjects may explain the inability of the heart to respond sufficiently to stimuli that activate the sympathetic nervous system.

Pearson et al. [10] speculated that autonomic dysregulation could alter the autonomic tone in SCA patients and may therefore exacerbate pain episodes by increasing peripheral vasoconstriction. Moreover, the sudden deaths of some SCA patients could be partly related to cardiovascular autonomic nervous dysfunction [11]. In patients with many forms of anemia, the decreased ANS activity and impaired sympatho-vagal balance has been attributed to chronic anemia. However, frequent-crisis SCA patients had lower parasympathetic activity than infrequent-crisis SCA patients, even though both groups exhibited a similar low hemoglobin concentration and hematocrit levels [14].

In rats, chronic intermittent hypoxia causes significant cell loss in the nucleus ambiguus, a structure from which several vagal efferent axons innervate ganglionated plexuses in the dorsal surface of cardiac atria, which in turn may have functional roles in cardiac regulation [36]. In addition, chronic intermittent hypoxia alters the structure of cardiac ganglia, resulting in the reorganization of vagal efferent projections to the cardiac ganglia [37]. Subclinical microvaso-occlusive and intermittent subclinical episodes of ischemia could contribute to the cardiovascular autonomic dysfunction observed in SCA [11]. The characteristic widespread vascular occlusions in SCA that cause chronic hypoxia can involve virtually every organ of the body, including the heart [38]. Moreover, cerebral thinning observed in patients with SCA may contribute to abnormalities of autonomic functions, and a hypersensitivity of the ANS responses to hypoxia may also be caused by the irregularities of brain function [39].

The results of the present study show that there was no significant difference between SCA patients and healthy subjects in pulse wave amplitudes in the basal state. It has previously been reported that increased peripheral blood flow occurs during the steady state with a periodic microcirculatory flow pattern, which may compensate for alterations in red blood cell rheology. The arteriolar diameter increases during the vaso-occlusive crisis compared to the intervals between the crises [7]. However, during the post-occlusion hyperemia test, SCA patients had small pulse wave amplitudes. This difference probably reflects the increased endothelial damage caused by repeated insults. The increase in pulse amplitude by hyperemia reflects a complex vascular response to ischemia [40,41]. Selvaraj et al. [42] reported that the PPG amplitude response reflected the metabolic component reinforcing the later course of RH. The results of the present study are consistent with the data of other groups examining endothelial dysfunction in SCA. Patients with sickle cell anemia had severely impaired endothelial function during crises [43] and in a steady-state phase [6] as compared to health-matched controls. Other studies reported that the group of

patients who had more symptoms had a significantly lower RH index than the group that was not as symptomatic.

The hyperactive endothelium seen in SCA enhances red blood cell and neutrophil adhesion, resulting in a slowed blood flow and sickling in post-capillary venules, followed by vaso-occlusion and ischemia [44]. In addition, Suvimol et al. [39] demonstrated that autonomic dysregulation in subjects with SCA enhanced normal vasoconstriction reflexes. Global vasoconstriction increases microvascular transit time and may promote the entrapment of sickle cells in the microvasculature, making vaso-occlusion more likely [31].

Many articles have reported an alteration of ANS activity in response to RH in patients with SCA. However, these papers did not assess dynamic changes in the ANS during the RH maneuver. The dynamics of ANS activation during RH may provide valuable information for evaluating the cardiovascular autonomic neuropathic that may be present in sickle cell disease. The results of the present study demonstrate that both ANS activity and PPG amplitude are impaired in patients with SCA; thus, the autonomic and endothelial status in SCA patients can be closely evaluated by applying the techniques detailed herein. Further studies are clearly warranted to clarify the relationship between altered ANS activity and SCA severity. A limitation of our study was the small sample size. This was, however, comparable to many papers concerning ANS activity in the literature.

5. Conclusions

Vasodilatory function, as measured by PPG, was lower in SCA patients compared to healthy subjects. Moreover, a cardiovascular autonomic imbalance was present in SCA patients with high sympathetic and low parasympathetic activity in the basal state and a poor response of the sympathetic nervous system to RH. An early cardiovascular sympathetic activation (10 s) and vasodilatory function in response to RH were impaired in SCA patients.

Author Contributions: Conceptualization, E.L.-G., A.A.V.-C., R.C.-E., M.P.-R., A.A.F.-G., I.C.-C., A.P.-S., P.C., M.E.S.-H. and G.A.M.-B.; methodology, E.L.-G., R.C.-E., M.P.-R., A.A.F.-G., P.C., M.E.S.-H. and G.A.M.-B.; software, R.C.-E. and A.P.-S.; validation, A.A.F.-G., I.C.-C. and G.A.M.-B.; formal analysis, E.L.-G., R.C.-E., A.P.-S., P.C., M.E.S.-H. and G.A.M.-B.; investigation, E.L.-G., A.A.V.-C., M.P.-R., I.C.-C. and M.E.S.-H.; resources, R.C.-E., M.E.S.-H. and G.A.M.-B.; data curation, M.P.-R., I.C.-C. and M.E.S.-H.; writing—original draft preparation, E.L.-G., A.A.V.-C. and M.E.S.-H.; writing—review and editing, R.C.-E., M.P.-R., A.A.F.-G., I.C.-C., A.P.-S., P.C. and G.A.M.-B.; visualization, A.A.V.-C., R.C.-E. and G.A.M.-B.; supervision, A.A.F.-G., I.C.-C., A.P.-S., P.C. and G.A.M.-B.; project administration, M.P.-R., A.A.F.-G., I.C.-C. and M.E.S.-H.; funding acquisition, M.E.S.-H. and G.A.M.-B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by “Apoyo a la Publicaciones Open Acces de la Universidad de las Americas de Chile”, “Dirección de Investigaciones de la Universidad Católica de la Santísima Concepción” and “Dirección de Investigaciones de la Universidad Adventista de Chile”.

Institutional Review Board Statement: Comité de Ética de la Facultad de medicina de la Universidad de Ciencias Médicas de Santiago de Cuba.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Voskaridou, E.; Christoulas, D.; Terpos, E. Sickle-cell disease and the heart: Review of the current literature. *Br. J. Haematol.* **2012**, *157*, 664–673. Available online: <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2141.2012.09143.x> (accessed on 25 December 2022). [[CrossRef](#)]
2. Couque, N.; Girard, D.; Ducrocq, R.; Boizeau, P.; Haouari, Z.; Missud, F.; Holvoet, L.; Ithier, G.; Belloy, M.; Odièvre, M.-H.; et al. Improvement of medical care in a cohort of newborns with sickle-cell disease in North Paris: Impact of national guidelines. *Br. J. Haematol.* **2016**, *173*, 927–937. Available online: <https://onlinelibrary.wiley.com/doi/abs/10.1111/bjh.14015> (accessed on 25 December 2022). [[CrossRef](#)]

3. Ware, R.E.; Montalembert, M.; Tshilolo, L.; Abboud, M.R. Sick cell disease. *Lancet* **2017**, *390*, 311–323. [CrossRef]
4. Cataldo, G.; Rajput, S.; Gupta, K.; Simone, D.A. Sensitization of nociceptive spinal neurons contributes to pain in a transgenic model of sickle cell disease. *Pain* **2015**, *156*, 722–730. Available online: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4366346/> (accessed on 25 December 2022). [CrossRef] [PubMed]
5. Coates, T.D.; Chalacheva, P.; Zeltzer, L.; Khoo, M.C.K. Autonomic nervous system involvement in sickle cell disease. *Clin. Hemorheol. Microcirc.* **2018**, *68*, 251–262. Available online: <https://content.iospress.com/articles/clinical-hemorheology-and-microcirculation/ch189011> (accessed on 25 December 2022). [CrossRef]
6. Belhassen, L.; Pelle, G.; Sediame, S.; Bachir, D.; Carville, C.; Bucherer, C.; Lacombe, C.; Galacteros, F.; Adnot, S. Endothelial dysfunction in patients with sickle cell disease is related to selective impairment of shear stress-mediated vasodilation. *Blood* **2001**, *97*, 1584–1589. [CrossRef]
7. Charlot, K.; Moeckesch, B.; Jumet, S.; Romana, M.; Waltz, X.; Divialle-Doumdo, L.; Hardy-Dessources, M.-D.; Petras, M.; Tressières, B.; Pichon, A.; et al. Physical activity level is not a determinant of autonomic nervous system activity and clinical severity in children/adolescents with sickle cell anemia: A pilot study. *Pediatr. Blood Cancer* **2015**, *62*, 1962–1967. Available online: <https://onlinelibrary.wiley.com/doi/abs/10.1002/pbc.25604> (accessed on 25 December 2022). [CrossRef]
8. Charlot, K.; Hierso, R.; Lemonne, N.; Romana, M.; Tressières, B.; Lalanne-Mistrih, M.L.; Etienne-Julan, M.; Tarer, V.; Ferracci, S.; Hardy-Dessources, M.; et al. Changes in autonomic nervous activity during vaso-occlusive crisis in patients with sickle cell anaemia. *Br. J. Haematol.* **2017**, *177*, 484–486. Available online: <https://onlinelibrary.wiley.com/doi/abs/10.1111/bjh.14064> (accessed on 25 December 2022). [CrossRef]
9. Inamo, J.; Connes, P.; Barthélémy, J.C.; Dan, V.; Coates, T.; Loko, G. Pulmonary hypertension does not affect the autonomic nervous system dysfunction of sickle cell disease. *Am. J. Hematol.* **2009**, *84*, 311–312. Available online: <https://onlinelibrary.wiley.com/doi/abs/10.1002/ajh.21377> (accessed on 25 December 2022). [CrossRef] [PubMed]
10. Pearson, S.R.; Alkon, A.; Treadwell, M.; Wolff, B.; Quirolo, K.; Boyce, W.T. Autonomic reactivity and clinical severity in children with sickle cell disease. *Clin. Auton. Res.* **2005**, *15*, 400–407. [CrossRef] [PubMed]
11. Mestre, J.C.R.; Hernández, A.; Agramonte, O.; Hernández, P. Cardiovascular autonomic dysfunction in sickle cell anemia: A possible risk factor for sudden death? *Clin. Auton. Res.* **1997**, *7*, 121–125. [CrossRef] [PubMed]
12. Alexy, T.; Sangkatumvong, S.; Connes, P.; Pais, E.; Tripette, J.; Barthelemy, J.C.; Fisher, T.; Meiselman, H.; Khoo, M.; Coates, T. Sick cell disease: Selected aspects of pathophysiology. *Clin. Hemorheol. Microcirc.* **2010**, *44*, 155–166. Available online: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2910781/> (accessed on 25 December 2022). [CrossRef] [PubMed]
13. Sangkatumvong, S.; Coates, T.D.; Khoo, M.C. Abnormal autonomic cardiac response to transient hypoxia in sickle cell anemia. *Physiol. Meas.* **2008**, *29*, 655–668. Available online: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2956125/> (accessed on 25 December 2022). [CrossRef]
14. Nebor, D.; Bowers, A.; Hardy-Dessources, M.D.; Knight-Madden, J.; Romana, M.; Reid, H.; Barthelemy, J.-C.; Cumming, V.; Hue, O.; Elion, J.; et al. Frequency of pain crises in sickle cell anemia and its relationship with the sympatho-vagal balance, blood viscosity and inflammation. *Haematologica* **2011**, *96*, 1589–1594. Available online: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3208675/> (accessed on 25 December 2022). [CrossRef] [PubMed]
15. Serhiyenko, V.A.; Serhiyenko, A.A. Cardiac autonomic neuropathy: Risk factors, diagnosis and treatment. *World J. Diabetes* **2018**, *9*, 1–24. Available online: <http://www.wjnet.com/1948-9358/full/v9/i1/1.htm> (accessed on 23 February 2022). [CrossRef]
16. Spallone, V.; Ziegler, D.; Freeman, R.; Bernardi, L.; Frontoni, S.; Pop-Busui, R.; Stevens, M.; Kempler, P.; Hilsted, J.; Tesfaye, S.; et al. Cardiovascular autonomic neuropathy in diabetes: Clinical impact, assessment, diagnosis, and management. *Diabetes Metab. Res. Rev.* **2011**, *27*, 639–653. Available online: <https://onlinelibrary.wiley.com/doi/abs/10.1002/dmrr.1239> (accessed on 23 February 2022). [CrossRef]
17. Zhang, Z.; Ma, Y.; Fu, L.; Li, L.; Liu, J.; Peng, H.; Jiang, H. Combination of Composite Autonomic Symptom Score 31 and Heart Rate Variability for Diagnosis of Cardiovascular Autonomic Neuropathy in People with Type 2 Diabetes. *J. Diabetes Res.* **2020**, *2020*, 5316769. Available online: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7648703/> (accessed on 23 February 2022). [CrossRef]
18. Bulte, C.S.E.; Keet, S.W.M.; Boer, C.; Bouwman, R.A. Level of agreement between heart rate variability and pulse rate variability in healthy individuals. *Eur. J. Anaesthesiol.* **2010**, *28*, 34–3827. Available online: https://journals.lww.com/ejanaesthesiology/Fulltext/2011/01000/Level_of_agreement_between_heart_rate_variability.8.aspx (accessed on 25 December 2022). [CrossRef]
19. Gil, E.; Orini, M.; Bailón, R.; Vergara, J.M.; Mainardi, L.; Laguna, P. Photoplethysmography pulse rate variability as a surrogate measurement of heart rate variability during non-stationary conditions. *Physiol. Meas.* **2010**, *31*, 1271. [CrossRef]
20. Lu, G.; Yang, F.; Taylor, J.A.; Stein, J.F. A comparison of photoplethysmography and ECG recording to analyse heart rate variability in healthy subjects. *J. Med. Eng. Technol.* **2009**, *33*, 634–641. [CrossRef]
21. Schäfer, A.; Vagedes, J. How accurate is pulse rate variability as an estimate of heart rate variability?: A review on studies comparing photoplethysmographic technology with an electrocardiogram. *Int. J. Cardiol.* **2013**, *166*, 15–29. Available online: [https://www.internationaljournalofcardiology.com/article/S0167-5273\(12\)00326-9/fulltext](https://www.internationaljournalofcardiology.com/article/S0167-5273(12)00326-9/fulltext) (accessed on 25 December 2022). [CrossRef] [PubMed]
22. Posada-Quintero, H.F.; Delisle-Rodríguez, D.; Cuadra-Sanz, M.B.; de la Vara-Prieto, R.R.F. Evaluation of pulse rate variability obtained by the pulse onsets of the photoplethysmographic signal. *Physiol. Meas.* **2013**, *34*, 179. [CrossRef]

23. Kandhai-Ragunath, J.J.; Jørstad, H.T.; de Man, F.H.; Peters, R.J.; vonBürgelen, C. Approaches for non-invasive assessment of endothelial function: Focus on peripheral arterial tonometry. *Neth. Heart J.* **2013**, *21*, 214–218. Available online: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3636344/> (accessed on 23 February 2022). [CrossRef] [PubMed]
24. Shimada, S.; Todoki, K.; Omori, Y.; Toyama, T.; Matsuo, M.; Wada-Takahashi, S.; Takahashi, S.-S.; Lee, M.-C. Contribution of nitrenergic nerve in canine gingival reactive hyperemia. *J. Clin. Biochem. Nutr.* **2015**, *56*, 98–104. Available online: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4345180/> (accessed on 22 February 2022). [CrossRef] [PubMed]
25. Krishnan, A.; Lucassen, E.B.; Hogeman, C.; Blaha, C.; Leuenberger, U.A. Effects of Limb Posture on Reactive Hyperemia. *Eur. J. Appl. Physiol.* **2011**, *111*, 1415–1420. Available online: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3285391/> (accessed on 22 February 2022). [CrossRef]
26. Schreuder, T.H.A.; Green, D.J.; Hopman, M.T.E.; Thijssen, D.H.J. Acute impact of retrograde shear rate on brachial and superficial femoral artery flow-mediated dilation in humans. *Physiol. Rep.* **2014**, *2*, e00193. Available online: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3967676/> (accessed on 22 February 2022). [CrossRef]
27. Short, K.R.; Blackett, P.R.; Gardner, A.W.; Copeland, K.C. Vascular health in children and adolescents: Effects of obesity and diabetes. *Vasc. Health Risk Manag.* **2009**, *5*, 973–990. Available online: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2788602/> (accessed on 22 February 2022).
28. Simón, C.A.P.; Allen, P.D.J. Nuevos modelos conceptual y matemático para el contorno de la onda de volumen de pulso arterial. *Rev. Cuba. Investig. Bioméd.* **2011**, *30*, 487–500. Available online: <http://scielo.sld.cu/pdf/ibi/v30n4/ibi07411.pdf> (accessed on 25 December 2022).
29. Malik, M. Heart rate variability: Standards of measurement, physiological interpretation, and clinical use: Task force of the European Society of Cardiology and the North American Society for Pacing and Electrophysiology. *Circulation* **1996**, *93*, 1043–1065. [CrossRef]
30. Torres-Leyva, M.; Carrazana-Escalona, R.; Ormigó-Polo, L.E.; Ricardo-Ferro, B.T.; López-Galán, E.; Ortiz-Alcolea, L.; Sánchez-Hechavarría, M.E. Cardiovascular autonomic response during the Cuban dynamic weight-bearing test. *CorSalud* **2019**, *11*, 1–10. Available online: <http://www.revcorsalud.sld.cu/index.php/cors/article/view/342/842> (accessed on 25 December 2022).
31. Khaleel, M.; Puliyeel, M.; Shah, P.; Sunwoo, J.; Kato, R.M.; Chalacheva, P.; Thuptimjang, W.; Detterich, J.; Wood, J.C.; Tsao, J.; et al. Individuals with sickle cell disease have a significantly greater vasoconstriction response to thermal pain than controls and have significant vasoconstriction in response to anticipation of pain. *Am. J. Hematol.* **2017**, *92*, 1137–1145. Available online: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5880319/> (accessed on 25 December 2022). [CrossRef] [PubMed]
32. Hedreville, M.; Charlot, K.; Waltz, X.; Sinnaph, S.; Lemonne, N.; Etienne-Julan, M.; Soter, V.; Hue, O.; Hardy-Dessources, M.-D.; Barthélémy, J.-C.; et al. Acute Moderate Exercise Does Not Further Alter the Autonomic Nervous System Activity in Patients with Sickle Cell Anemia. *PLoS ONE* **2014**, *9*, e95563. Available online: <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0095563> (accessed on 25 December 2022). [CrossRef] [PubMed]
33. López-Galán, E.; Andreu-Heredia, A.; Carrazana-Escalona, R.; Querts-Menéndez, O.; García-Naranjo, J.C.; Lazo-Herrera, L.A.; Muñoz-Bustos, G.A.; Albarrán-Torres, F.A.; Sánchez-Hechavarría, M.E. La activación precoz del reflejo simpático cardiovascular es independiente del tiempo de oclusión durante la hiperemia reactiva. *CorSalud* **2022**, *14*, 164–172. Available online: <https://revcorsalud.sld.cu/index.php/cors/article/view/754> (accessed on 25 December 2022).
34. Boushel, R. Muscle metaboreflex control of the circulation during exercise. *Acta Physiol.* **2010**, *199*, 367–383. Available online: <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1748-1716.2010.02133.x> (accessed on 25 December 2022). [CrossRef] [PubMed]
35. Kaur, J.; Spranger, M.D.; Hammond, R.L.; Krishnan, A.C.; Alvarez, A.; Augustyniak, R.A.; O’Leary, D.S. Muscle metaboreflex activation during dynamic exercise evokes epinephrine release resulting in β 2-mediated vasodilation. *Am. J. Physiol.-Heart Circ. Physiol.* **2015**, *308*, H524–H529. Available online: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4346770/> (accessed on 25 December 2022). [CrossRef] [PubMed]
36. Ai, J.; Epstein, P.N.; Gozal, D.; Yang, B.; Wurster, R.; Cheng, Z.J. Morphology and topography of nucleus ambiguus projections to cardiac ganglia in rats and mice. *Neuroscience* **2007**, *149*, 845–860. Available online: <https://www.sciencedirect.com/science/article/pii/S0306452207009530> (accessed on 25 December 2022). [CrossRef] [PubMed]
37. Lin, M.; Ai, J.; Li, L.; Huang, C.; Chapleau, M.W.; Liu, R.; Gozal, D.; Wead, W.B.; Wurster, R.D.; Cheng, Z. Structural remodeling of nucleus ambiguus projections to cardiac ganglia following chronic intermittent hypoxia in C57BL/6J mice. *J. Comp. Neurol.* **2008**, *509*, 103–117. Available online: <https://onlinelibrary.wiley.com/doi/abs/10.1002/cne.21732> (accessed on 25 December 2022). [CrossRef]
38. Ahmed, S.; Siddiqui, A.K.; Sadiq, A.; Shahid, R.K.; Patel, D.V.; Russo, L.A. Echocardiographic abnormalities in sickle cell disease. *Am. J. Hematol.* **2004**, *76*, 195–198. Available online: <https://onlinelibrary.wiley.com/doi/abs/10.1002/ajh.20118> (accessed on 30 January 2023). [CrossRef]
39. Sangkatumvong, S.; Khoo, M.C.K.; Kato, R.; Detterich, J.A.; Bush, A.; Keens, T.G.; Meiselman, H.J.; Wood, J.C.; Coates, T.D. Peripheral Vasoconstriction and Abnormal Parasympathetic Response to Sighs and Transient Hypoxia in Sickle Cell Disease. *Am. J. Respir. Crit. Care Med.* **2011**, *184*, 474–481. Available online: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3175540/> (accessed on 25 December 2022). [CrossRef]
40. Thijssen, D.; Atkinson, C.; Ono, K.; Sprung, S.; Spence, A.; Pugh, C.; Green, D. Sympathetic nervous system activation, arterial shear rate, and flow-mediated dilation. *J. Appl. Physiol.* **2014**, *116*, 1300–1307. Available online: <https://journals.physiology.org/doi/full/>

[10.1152/jappphysiol.00110.2014?rfr_dat=cr_pub++0pubmed&url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org](https://doi.org/10.1152/jappphysiol.00110.2014?rfr_dat=cr_pub++0pubmed&url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org) (accessed on 25 December 2022). [[CrossRef](#)]

41. Yoshida, M.; Tomiyama, H.; Shiina, K.; Odaira, M.; Yamashina, A. The difference of the influence of autonomic nervous activation caused by reactive hyperemia on two different endothelial function tests. *Eur. Heart J.* **2013**, *34* (Suppl. S1), P1436. [[CrossRef](#)]
42. Selvaraj, N.; Jaryal, A.K.; Santhosh, J.; Anand, S.; Deepak, K.K. Monitoring of reactive hyperemia using photoplethysmographic pulse amplitude and transit time. *J. Clin. Monit. Comput.* **2009**, *23*, 315–322. [[CrossRef](#)]
43. Blum, A.; Yeganeh, S.; Peleg, A.; Vigder, F.; Kryuger, K.; Khatib, A.; Khazim, K.; Dauerman, H. Endothelial Function in Patients with Sickle Cell Anemia during and After Sickle Cell Crises. *J. Thromb. Thrombolysis* **2005**, *19*, 83–86. [[CrossRef](#)] [[PubMed](#)]
44. Ansari, J.; Gavins, F.N.E. Ischemia-Reperfusion Injury in Sickle Cell Disease: From Basics to Therapeutics. *Am. J. Pathol.* **2019**, *189*, 706–718. Available online: [https://ajp.amjpathol.org/article/S0002-9440\(18\)30622-9/fulltext](https://ajp.amjpathol.org/article/S0002-9440(18)30622-9/fulltext) (accessed on 25 December 2022). [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.