disminuir las dosis. Según la experiencia de nuestro centro y otras series, en estos pacientes el fracaso del tratamiento médico es muy frecuente, bien desde el inicio o al reducir las dosis de corticoides, lo que para la mayoría deja como única opción la pericardiectomía[4,5]. Es por tanto de especial interés el hecho de que, en nuestro caso, el intenso esquema terapéutico inmunosupresor, así como la guía del mismo mediante ecografía y resonancia magnética cardiaca para comprobar su eficacia, logró evitar la pericardiectomía, que en estos pacientes tiene un alto riesgo y una moderada efectividad. El precio a pagar por las altas dosis de corticoides utilizadas fueron las complicaciones infecciosas.

En un paciente con pericarditis efusiva con datos de infección bacteriana concomitantes y con otro foco infeccioso siempre se debe incluir la pericarditis bacteriana dentro del diagnóstico diferencial inicial y valorar la realización de pericardiocentesis urgente incluso antes de probar con la antibioterapia inicial. No obstante, en nuestro caso el derrame pericárdico tenía características muy heterogéneas con aspecto organizado, por lo que se desestimó la pericardiocentesis y se optó directamente por una ventana pericárdica.

Dada la escasa evidencia científica sobre el tratamiento terapéutico de la pericarditis efusivo-constrictiva secundaria a esta etiología tan agresiva y a la morbimortalidad asociada al tratamiento quirúrgico de la misma, nuestra experiencia sugiere el valor del tratamiento médico intenso guiado por la información proporcionada por las diferentes técnicas de imagen y la evolución clínica.

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Expansion of CD4^{+}CD28^{NULL} T lymphocytes in patients with focal epicardial spasm. A potential novel pathogenetic role

Expansión de linfocitos T CD4^{+}CD28^{NULL} en pacientes con vasoespasmo focal: un potencial y novedoso rol patogenético

To the Editor,

Patients with angina pectoris despite angiographically unobstructed coronary arteries represent a diagnostic and therapeutic challenge. Coronary artery spasm (CAS) is an established cause of chest pain in patients with stable angina and unobstructed coronary arteries. Recent data have shown that approximately 50% of patients undergoing diagnostic coronary angiography for the assessment of typical chest pain have unobstructed coronary arteries and that CAS can be found in approximately 60% of such patients.[1] These functional coronary vasomotor disorders may occur at the focal or diffuse epicardial level or in the microvasculature. However, the underlying pathomechanisms are still partially understood and data on circulating immune cells contributing to CAS is scarce. It has been shown that an unusual subset of T lymphocytes, characterized by the lack of CD28 receptor expression (CD4^{+}CD28^{NULL} T cells) are expanded in patients with acute coronary syndrome.[2] It is believed that this T-cell subset can exert high proinflammatory (e.g. by high production of interferon-γ) and cytolytic properties leading to endothelial dysfunction and promotion of vascular inflammation. To the best of our knowledge, the role of CD4^{+}CD28^{NULL} T cells has not been investigated in patients with vasospastic angina. Thus, the aim of this study is to assess whether expansion of CD4^{+}CD28^{NULL} T cells is associated with CAS.

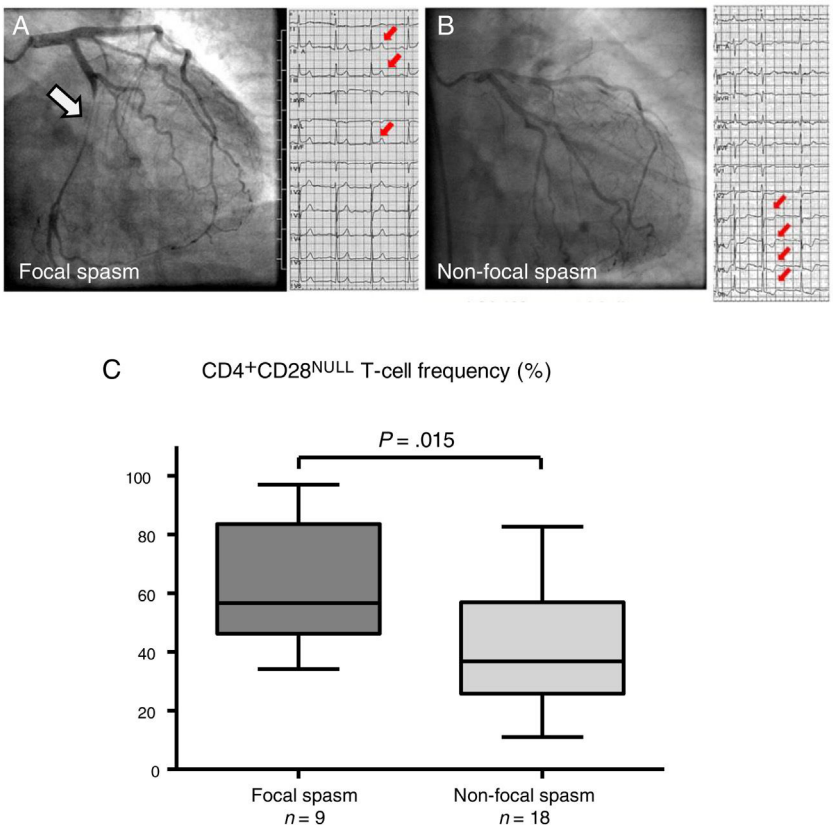

Fig. 1 – (A) Left coronary artery angiograms and electrocardiograms (ECGs) of a representative patient with epicardial focal spasm: In the left circumflex artery epicardial focal spasm (white arrow) was provoked by 100 μg intracoronary (i.c.) acetylcholine (ACh) with concomitant ST-segment elevation (small arrows) and reproduction of the usual symptoms. (B) Left coronary artery angiograms and ECGs in a representative patient with non-focal (microvascular) spasm: During 100 μg i.c. ACh infusion the patient showed reproduction of the usual symptoms with concomitant ST-segment depression (small arrows), but no relevant epicardial vasoconstriction. (C) Statistical analysis revealed that patients with focal coronary spasm had a significantly higher frequency of $CD4^{+}CD28^{NULL}$ T cells compared to patients without focal spasm. Data are presented as box plots displaying medians, 25th and 75th percentiles (boxes) with Min/Max whiskers. Angiograms and ECGs in this figure have been previously published in Ong et al.[3]

To address this issue 27 consecutive patients (19 men, age 57 ± 15 years) with stable angina despite angiographically unobstructed coronary arteries (< 50% stenosis) who underwent intracoronary acetylcholine (ACh) testing for the diagnosis of CAS were assessed between November 2014 and June 2015 within a substudy of the ACOVA trial.[1] All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all individual participants included in the study. For inclusion in the study, patients had to have symptoms suggestive of coronary artery disease (chest pain or dyspnoea, with or without occasional attacks of resting chest pain) with a positive result in the ACh-test. Patients with chronic obstructive pulmonary disease, cardiomyopathy, severe valvular heart disease, myocardial infarction within the last 3 months, or chronic inflammatory conditions (e.g. rheumatoid arthritis, lupus, etc.) were not included in the study. The ACh-test was performed directly after diagnostic coronary angiography according to a standardized protocol.[3] Patients were classified according to the presence of focal (diameter reduction ≥ 90% in only one segment) or diffuse (diameter reduction ≥ 90% in ≥ 2 adjacent coronary segments) "epicardial coronary artery spasm" or "microvascular spasm" (no relevant changes of epicardial vessel diameter < 90%; see also Fig. 1). Because other studies have described a worse clinical outcome associated with focal coronary artery spasm in vasospastic angina,[4] the patients in the present study were divided into two groups. Group 1 comprised patients with focal epicardial coronary spasm (focal spasm) and group 2 comprised patients with diffuse or microvascular spasm (non-focal spasm). In addition, the following information was recorded in every patient: gender, age at the date of the assessment, clinical presentation and traditional cardiovascular risk factors.

Expansion of $CD4^{+}CD28^{NULL}$ T cells in peripheral venous blood (EDTA whole blood samples) was directly analyzed and evaluated on the same day of blood collection on the flow cytometer BD FACSVerse using BD FACSuite Software (BD Biosciences, Germany). Non-specific, false-positive antibody staining was blocked using FcR-blocking reagent (Human BD Fc Block, BD Biosciences) for 10 min at room temperature. For each patient a volume of 50 μL blood sample per tube was incubated with antibodies against CD4 (PerCP

Table 1 – Clinical characteristics of the study patients.

	All patients ($n=27$)	Group 1 Focal spasm ($n=9$ [33%])	Group 2 Non-focal spasm ($n=18$ [67%])	P
Men	19 (70%)	8 (89%)	11 (61%)	.201
Age (years, mean ± SD)	57 ± 15	58 ± 16	56 ± 15	.814
Clinical presentation				
Resting angina only	5 (19%)	3 (33%)	2 (11%)	.295
Predominantly resting angina	4 (15%)	2 (22%)	2 (11%)	.582
Mix of resting and effort angina	6 (22%)	2 (22%)	4 (22%)	1.0
Predominantly effort angina	1 (4%)	0 (0%)	1 (6%)	1.0
Effort angina only	4 (15%)	0 (0%)	4 (22%)	.268
Dyspnoea only	5 (19%)	1 (11%)	4 (22%)	.636
No angina	2 (7%)	1 (11%)	1 (6%)	1.0
Cardiovascular risk factors				
Hypertension	14 (52%)	7 (78%)	7 (39%)	.103
Diabetes mellitus type II	7 (26%)	4 (44%)	3 (17%)	.175
Hypercholesterolemia	18 (67%)	4 (44%)	14 (78%)	.109
Smoking	11 (41%)	5 (56%)	6 (33%)	.411
Obesity	8 (30%)	1 (11%)	7 (39%)	.201
Family history for cardiovascular events	12 (44%)	3 (33%)	9 (50%)	.683

Data are expressed as no. (%) or mean ± standard deviation.

mouse anti-human CD4 Clone SK3, #345770; BD Biosciences) and CD28 (BV421 mouse anti-human CD28 Clone CD28.2, #562613; BD Biosciences) for 20 min, followed by a lysing step (Lysing buffer, BD Pharmingen; #555899) for 15 min (both steps were carried out at room temperature protected from light according to the LNW method; lyse no wash method). For quantification of the measured events BD Trucount tubes were used (BD BioSciences; #340334) and verified with the implemented Flow Sensor. Autofluorescence- and isotype-controls were used to discriminate specific and non-specific antibody binding. Gates with the cells of interest were defined with the help of the single staining and the FMO control (Fluorescence Minus One) by exchanging the CD28-BV421 antibody with the corresponding isotype control IgG1-BV421. Acquisition was performed until a total number of 5000 BD Trucount bead were analyzed and the frequency of $CD4^+CD28^{NULL}$ T cells was expressed as percentage of total $CD4^+$ T cells.

Results are expressed as mean ± standard deviation (SD). For statistical analysis of $CD4^+CD28^{NULL}$ T cells the Student *t* test was used to compare normally distributed continuous variables, while Fisher's exact test was used for categorical variables. A two-tailed $P < .05$ was considered significant. Statistical data analysis was performed using SPSS 23.0 (IBM, USA).

The ACh-test revealed epicardial spasm in 13 patients, 9 of them had focal and 4 had diffuse spasm. The remaining 14 patients had coronary microvascular spasm on ACh-testing. The clinical characteristics of the study patients are summarized in Table 1. Although 19 of 27 patients in our study cohort were men (70%), the proportion of male patients in group 1 (focal spasm) and group 2 (non-focal spasm) was not significantly different, 89% and 61%, respectively ($P = .201$). The comparison of age, clinical presentation (type of anginal symptoms) and cardiovascular risk factors revealed no statistically significant differences between patients with focal coronary spasm compared to patients with diffuse or microvascular spasm. As shown in Fig. 1, the frequency of $CD4^+CD28^{NULL}$ T cells assessed via flow cytometry was significantly higher in patients with focal coronary spasm in comparison to the rest of the cohort ($P = .015$).

In the present study we have shown for the first time that patients with focal epicardial spasm have a significantly greater expansion of $CD4^+CD28^{NULL}$ T cells, an unusual subset of $CD4^+$ T cells known to have high proinflammatory and cytotoxic properties, compared to patients without focal spasm. These findings may explain, at least in part, why other studies have described a worse clinical outcome associated with focal coronary spasm.[4] This is further supported by other studies showing that $CD4^+CD28^{NULL}$ T cell frequency is an independent predictor of future acute coronary events in patients with unstable angina.[5] A caveat may lie in the fact that $CD4^+CD28^{NULL}$ T cell expansion is related to age, a known marker for T cell senescence.[6] However, the mean age in our cohort did not differ significantly between focal spasm compared to the rest of the cohort (58 ± 16 years vs 56 ± 15 years; $P = .814$). Therefore we can exclude that differences in age between the 2 groups might have influenced $CD4^+CD28^{NULL}$ T cell frequency in our study. Furthermore, to rule out a possible contribution of chronic inflammatory disorders to $CD4^+CD28^{NULL}$ T cell expansion, patients with chronic inflammatory conditions (e.g. rheumatoid arthritis) were excluded from the present study. The frequency of patients with diabetes was comparable between the 2 groups suggesting that this did not influence the differences in $CD4^+CD28^{NULL}$ T cell frequency significantly (Table 1). A limitation of this study is the small patient cohort ($n = 27$). A lager study population is necessary to confirm the results and to assess the clinical relevance by examination of $CD4^+CD28^{NULL}$ T cell frequency in correlation with clinical outcome in patients with focal coronary spasm. Mechanistic in vitro studies suggest that the cytotoxic and inflammatory properties of $CD4^+CD28^{NULL}$ T cells depend on their receptor profile, for example receptors of the killer immunoglobulin-like receptor family.[2] Therefore further studies should characterize the functional properties and receptor profile of $CD4^+CD28^{NULL}$ T cells expanded in patients with focal coronary spasm in more detail.

In conclusion, in the present study patients with focal epicardial spasm show a significantly greater expansion of $CD4^{+}CD28^{NULL}$ T cells compared to patients with other coronary vasomotor disorders. This underlines the role of an inflammatory pathomechanism especially in patients with focal epicardial spasm. Further larger clinical trials are necessary to clarify whether modulation of $CD4^{+}CD28^{NULL}$ T cells may represent a potential therapeutic target in the pathogenesis of CAS.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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