UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE MEDICINA

TESIS DOCTORAL

Medicina de precisión en la Inmunodeficiencia Variable Común

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Kissy Guevara Hoyer

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Madrid, 2020

“Si el hombre vive es porque cree en algo.”

LEV TOLSTÓI

“Quien no sabe lo que busca
no ve lo que encuentra.”
CLAUDE BERNARD

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ÍNDICE

ABREVIATURAS

APC. Célula presentadora de antígeno.
APRIL. Un ligando de inducción de proliferación.
BAFF-R. Factor activador de linfocitos B dependiente de la familia del receptor de factor de necrosis tumoral.
BCR. Receptor de la célula B.
CMV. *Citomegalovirus.*
CVID. Common variable immunodeficiency.
ESID. Sociedad Europea de Inmunodeficiencias.
fSCIg. Inmunoglobulina vía subcutánea facilitada.
GI. Gastrointestinales.
HLA. Antígeno leucocitario humano.
IDP. Inmunodeficiencia Primaria.
IDVC. Inmunodeficiencia Variable Común.
Ig. Inmunoglobulinas.
IgRT. Reemplazo con inmunoglobulinas.
IUIS. Recientemente la Unión Internacional de Sociedades Inmunológicas.
IVIG. Inmunoglobulina vía endovenosa.
κ. Cadena ligera de inmunoglobulinas libre en suero, kappa.
λ. Cadena ligera de inmunoglobulinas libre en suero, Lambda.
LIE. Linfocitos intraepiteliales.
LOCID. Inmunodeficiencia combinada de inicio tardío.
LPS. Lipopolisacáridos.
MHC. Complejo mayor de histocompatibilidad.
NGS. Secuenciación de nueva generación.
PAMPS. Patrones asociados a patógenos.
PBMC. Células mononucleares de sangre periférica.
PHA. Fitohemaglutinina.
PPV. Vacuna 23-valente de neumococo.
PWM. Mitógeno de hierba carmín.
QoL. Calidad de vida.
RR. Riesgo relativo.
RRTI. Infecciones recurrentes del tracto respiratorio inferior.
SCIG. Inmunoglobulina vía subcutánea.
sFLC. Cadenas ligeras de inmunoglobulinas libres en suero.
SHM. Hipermutación somática.
SUMA κ+ λ. La sumatoria de κ+ λ.
TACI. Activador transmembrana y modulador del calcio.
TC. Tomografía computarizada de alta resolución.
Th. T colaborador (helper).
TibV. Vacunas basadas en inmunidad entrenada.
TLR. Receptores de tipo toll.
TNF. Factor de necrosis tumoral.
TPH. Trasplante de progenitores hematopoyéticos.
T_{Reg}. Células T reguladoras.
TWEAK. Inductor débil de apoptosis TNF-like.
VISUAL. Variable Immunodeficiency Score Upfront Analytical Link.
WES. Secuenciación del exoma completo.
WGS. Secuenciación del genoma completo.

RESUMEN
SUMMARY

La inmunodeficiencia variable común (IDVC) es la inmunodeficiencia primaria (IDP) sintomática más frecuente, caracterizada por una elevada susceptibilidad a infecciones bacterianas recurrentes, así como un amplio perfil de expresión clínica con manifestaciones autoinmunes, inflamatorias y predisposición a malignidad. Su variabilidad clínica sugiere un defecto inmunoregulatorio complejo, con alteraciones tanto en la inmunidad innata como en la adaptativa. La caracterización genética ha cobrado especial interés relevante con implicación pronóstica y terapéutica. El primer y más importante escalón terapéutico es el reemplazo con inmunoglobulinas (IgRT), que si se inicia precozmente puede disminuir drásticamente el número y la gravedad de las infecciones. El pronóstico de los pacientes con IDVC está condicionado principalmente por la existencia de complicaciones infecciosas, así como por el desarrollo de malignidad.

El objetivo principal de este estudio fue establecer nuevos biomarcadores diagnósticos y pronósticos de la IDVC, así como determinar y validar una escala basada en la combinación de biomarcadores independientes con poder pronóstico de la IDVC, que posibilite un seguimiento y tratamiento más personalizados del paciente individual. En paralelo a esta línea de investigación, se pretende profundizar en el conocimiento de los mecanismos biológicos y fisiopatológicos implicados en la IDVC. Se adjuntan tres artículos originales que compilan los resultados correspondientes a los objetivos establecidos en esta tesis.

La primera sección de este trabajo de tesis consistió en la caracterización detallada de dos cohortes de pacientes con IDVC geográficamente diferentes y la aplicación de las clasificaciones y escalas clínicas establecidas para IDVC. Como biomarcador diagnóstico se propone, en primer lugar, la medición de las cadenas ligeras de inmunoglobulinas libres en suero (sFLC) como marcador diagnóstico diferencial de la IDVC frente otras inmunodeficiencia primarias y secundarias (IDS). Se propone así la suma de kappa y lambda (SUMA $\kappa+\lambda$) como un nuevo criterio diagnóstico de la IDVC, con un punto de corte de <16,7 mg/L, que presenta una sensibilidad del 92%, una especificidad del 75% y un VPN del 98% para el diagnóstico de IDVC frente a otras IDP, así como de IDS.

En cuanto a los marcadores pronósticos en la IDVC, por un lado, se propone el estudio de HLA de clases II en los pacientes con IDVC, como marcador pronóstico en el desarrollo de enfermedad autoinmune y enteropatía inexplicada, basados en la frecuencia de haplotipos de

HLA de clase II en nuestra cohorte, relacionados significativamente con mayor susceptibilidad de presentar enfermedad autoinmune. Se observó una asociación significativa entre la expresión de haplotipos de HLA de susceptibilidad de enfermedad celíaca y el desarrollo de manifestaciones GI no infecciosas ($p < 0.05$), asociación previamente no descrita. El estudio de activación de los linfocitos T $CD8^+$ en pacientes con IDVC se asoció con enfermedad autoinmune, detectándose en nuestro estudio la correlación inversa entre el aumento de HLA-DR^+ en la superficie de los linfocitos T $CD8^+$ y la expresión de $CD45RA^+$ en pacientes con enfermedad autoinmune, así como la asociación con la función supresora de T_{Reg} significativamente disminuida.

La clasificación de los patrones de sFLC en la IDVC se validaron como marcadores pronósticos de manifestaciones por fenotipos clínicos. Se observó una correlación entre el patrón $\kappa^-\lambda^+$ con mayor incidencia de infecciones respiratorias superiores e inferiores; el patrón $\kappa^-\lambda^-$ con enteropatía, esplenomegalia y bronquiectasias, hallazgos similares a estudios previos. Por otra parte, se propone la determinación y seguimiento de la alteración del cociente de sFLC κ/λ como marcador pronóstico de desarrollo de síndrome linfoproliferativo B en pacientes con IDVC.

Se ha desarrollado una escala pronóstica combinada para la IDVC: Variable Immunodeficiency Score Upfront Analytical Link (VISUAL), que es una de las aportaciones fundamentales de este trabajo. VISUAL utiliza 4 biomarcadores inmunológicos combinados de cribado (linfocitos B de memoria con cambio de isotipo, IgA, respuestas a anticuerpos específicas y linfocitos T $CD4^+$), no invasivos y fáciles de realizar de forma rutinaria en el momento del diagnóstico que permiten predecir precozmente la gravedad de manifestaciones clínicas en los pacientes con IDVC. VISUAL ≥10 puntos tiene una sensibilidad del 85% y un VPN del 77% de identificar al diagnóstico los casos graves según la escala de gravedad establecida, significativamente superior al porcentaje de linfocitos B de memoria con cambio de isotipo de forma aislada ($p=0.01$).

En cuanto a las aportaciones en el tratamiento de la IDVC, proponemos a las vacunas basadas en inmunidad entrenada como estrategia profiláctica en la IDVC con infecciones a pesar de IgG valle adecuada, basados en un estudio de prueba de concepto en 10 pacientes con IDVC e

infecciones respiratorias recurrentes (RRTI), que fueron tratados con la vacuna polibacteriana MV130. MV130 asoció una disminución significativa en la tasa de infección durante los 12 meses posteriores al inicio del tratamiento (p=0,006), en el uso de antibióticos (p=0,005) y en las visitas ambulatorias no programadas por infecciones (p=0,002), con una mejoría de la percepción global de la calidad de vida en más del 50% de los pacientes, con hasta 17% con respecto a la puntuación del score previo al tratamiento.

Por último, se plantean diferentes hipótesis para explicar la disminución de las sFLC en la IDVC, desde un reordenamiento alterado, a un ensamblaje o secreción de cadena ligera durante la ontogenia de linfocitos B ó bien a un defecto intrínseco de secreción de cadenas ligeras por parte de las células plasmáticas. Esta hipótesis apunta a un defecto específico en la diferenciación de las células plasmáticas, remontándose probablemente a un evento temprano crítico durante la diferenciación del linfocito B. Actualmente se esta llevando a cabo un proyecto para investigar estas hipótesis.

En resumen, este trabajo de tesis ha contribuido a la identificación de nuevos biomarcadores diagnósticos y pronósticos, así como a innovadoras opciones terapéuticas complementarias en la IDVC, así como proporcionar nueva evidencia de las alteraciones biológicas subyacentes en esta enfermedad, dejando abiertas nuevas líneas de investigación, así como la cuestión de si sería necesario revisar la clasificación de IDVC de acuerdo con estos resultados.

Common variable immunodeficiency (CVID) is the most frequent symptomatic primary immunodeficiency (PID), characterized by susceptibility to recurrent bacterial infections, as well as a broad clinical profile with autoimmune, inflammatory manifestations and predisposition to malignancy. The clinical variability of CVID suggests a complex immunoregulatory defect, with alterations in both innate and adaptive immunity. Genetic and molecular characterization has gained relevant interest nowadays. The immunoglobulin replacement (IgRT) is the most important therapeutic approach with prognostic implication in CVID. The main objective of this study was to establish new CVID diagnostic and prognostic biomarkers, as well as to determine and validate a scale based on the combination of independent biomarkers with potential prognostic role in CVID. In conjunction with this project, we intend to deepen our understanding of the biological and pathophysiological mechanisms involved in CVID. The results corresponding to the objectives established in this thesis are compiled in three original articles, which are attached to this work.

The first section of this thesis consisted of the detailed characterization of two geographically different cohorts of CVID patients, as well as, the application of the clinical classifications and scales established for this disease. As a diagnostic biomarker, the measurement of serum immunoglobulin free light chains (sFLC) is proposed as a differential diagnostic marker of CVID against other primary and secondary immunodeficiencies (SID). Thus, sum of kappa and lambda (SUM $\kappa + \lambda$) is proposed as a new diagnostic CVID criteria. Cut-off of 16.7 mg/L, giving the highest combination of sensitivity (92%), specificity (75%) and NPV (98%).

Regarding CVID prognostic markers, HLA class II determination is proposed as a prognostic marker in the development of autoimmune disease and unexplained enteropathy. This proposal is based on the significantly HLA class II haplotypes expression related to autoimmune disease in our cohort, as well as, the significant association between the expression of HLA haplotypes of celiac disease susceptibility with non-infectious GI manifestations ($p < 0.05$), association not described previously. $CD8^+$ T lymphocytes activation was associated with autoimmune disease in CVID patients. Our study detected an inverse correlation between the increase of HLA-DR^+ expression on $CD8^+$ T lymphocytes surface and $CD45RA^+$ expression, as well as, a significantly decreased in suppressive T_{Reg} function in patients with autoimmune disease.

Concerning sFLC patterns in CVID were validated as prognostic markers of the clinical phenotypes. $\kappa^{-}\lambda^{+}$ pattern presented a correlation with a higher incidence of upper and lower respiratory infections and $\kappa^{-}\lambda^{-}$ pattern was associated with enteropathy, splenomegaly and bronchiectasis, findings similar to previous studies. On the other hand, the determination and monitoring of κ/λ sFLC ratio is proposed as a prognostic marker for B lymphoproliferative syndrome in CVID patients.

A combined prognostic scale for CVID has been developed: Variable Immunodeficiency Score Upfront Analytical Link (VISUAL); one of the fundamental contributions of this work.
VISUAL uses 4 combined immunological screening biomarkers (class-switched memory B cell, IgA, specific antibody responses and $CD4^{+}$ T cells), characterized by being non-invasive and easy to perform routinely at the time of diagnosis that allow early prediction of the severity of clinical manifestations in patients with CVID. VISUAL ≥10 points has a sensitivity of 85% and a NPV of 77% to identify severe cases at diagnosis according to the established severity scale, significantly higher than the class-switched memory B cell in isolation ($p = 0.01$).

Regarding the contributions in the treatment of CVID. We propose trained immunity-based vaccine (TIbV) as a prophylactic strategy in CVID patients with infections despite adequate trough IgG levels, based on a proof-of-concept study in 10 CVID patients with recurrent respiratory infections (RRTI), who received the polybacterial preparation MV130. TIbV MV130 showed a significant decrease in infection rate ($p = 0.006$) throughout the 12 months after initiation of the treatment. A decrease in antibiotic use and unscheduled outpatient visits was observed ($p = 0.005$ and $p = 0.002$, respectively). Regarding the CVID QoL questionnaire, an overall decrease in the score by more than 50% was observed ($p < 0.05$) which demonstrated that patients experienced an improvement in their QoL.

Finally, different hypotheses to explain the decrease in sFLC in IDVC are proposed, since an altered rearrangement, to an assembly or secretion of light chain during the B lymphocytes ontogeny or to an intrinsic defect of light chain secretion by plasma cells. This hypothesis points to a specific defect in plasma cell differentiation, probably generated to a critical early event during B lymphocyte differentiation. Currently, a project is underway to investigate these hypotheses.

In summary, this thesis has contributed to the identification of new diagnostic and prognostic biomarkers, as well as innovative complementary therapeutic options in CVID. This study provides new evidence of the underlying biological alterations in this disease, providing new lines of research, as well as the question of whether it would be necessary to revise the IDVC classification according to these results.

INTRODUCCIÓN

1.Historia y Definición de la Inmunodeficiencia Variable Común

La inmunodeficiencia variable común (IDVC) es la inmunodeficiencia primaria (IDP) sintomática y heterogénea más frecuente (3). Se caracteriza por aumento de predisposición a infecciones bacterianas recurrentes, consecuencia de una baja producción de anticuerpos frente a patógenos (4). Puede manifestarse con amplio perfil de expresión clínica, desde mayor riesgo de autoinmunidad, enteropatía, manifestaciones inflamatorias, infiltración linfocítica policlonal, hasta predisposición a malignidades como linfoma y cáncer gástrico, entre otros (5). Se han descrito diversos genes envueltos en la patogénesis de la enfermedad, que afectan fundamentalmente al receptor del linfocito B (como TACI, BAFF-R,TWEAK, CD19 *complex*, IL21R, LRBA, ERK), moléculas relevantes en la coestimulación B-T (como CD27, ICOS, PI3K, Rac2, CARMA1/CAD11) o moléculas implicadas en activación linfocitaria (como las moléculas de la familia del TNF, CTLA-4, IP3, LCK, Vav1, ZAP70, TLRs) que contribuyen a un 15% de las causas de IDVC (4). Por consiguiente, en la mayoría de los casos el gen o genes responsables no ha sido identificado.

Charles Alderson Janeway, Jr. fue el primero en describir un caso de IDVC en 1953 (6). El caso correspondía a un varón de 39 años con infecciones respiratorias recurrentes, bronquiectasias y meningitis (7). Sin embargo, no fue hasta la década de 1990, en que se estableció una definición estándar para IDVC, con posterior desarrollo de criterios de diagnóstico, estableciendo la edad mínima de diagnóstico y la necesidad de excluir otras afecciones para describir la enfermedad (8). Originalmente, el nombre "variable" se utilizó para distinguir un grupo de hipogammaglobulinemias no clasificadas de inicio tardío, de las formas más severas y heredadas de agammaglobulinemia (9). Con el tiempo, ese término también se utilizó para aquellas formas de déficit primario de anticuerpos que se presentaron en la infancia tardía o en adultos (10).

Con el objetivo de definir mejor la heterogeneidad inherente de las características inmunológicas de la IDVC, la Organización Mundial de la Salud y la Sociedad Europea de Inmunodeficiencias (ESID) emitieron el primer consenso sobre los criterios de diagnóstico de la IDVC en un panel de expertos (11). El ensayo EUROclass fue diseñado para definir subgrupos de IDVC según el fenotipo de memoria B, basados en los esquemas de clasificación previos de París y Friburgo, respectivamente (12–14). Sin embargo, esta nueva clasificación no abarcaba todas las manifestaciones asociadas con la enfermedad.

La ESID ha revisado recientemente los criterios establecidos para el diagnóstico de IDVC **(Tabla 1)** basándose los criterios actuales en la presencia de hipogammaglobulinemia (disminución marcada en IgG e IgA con o sin disminución de IgM) asociado a infecciones recurrentes. Otros criterios mayores son la presencia de manifestaciones autoinmunes, enfermedad granulomatosa, linfoproliferación policlonal inexplicable e historia familiar de deficiencia de anticuerpos. Entre los criterios analíticos se debe considerar una baja producción de anticuerpos tras vacunación (y/o ausencia de isohemaglutininas), estudio de fenotipo de linfocitos B con recuento disminuido de células B de memoria con cambio de isotipo y ausencia de deficiencia profunda de células T (recuento de linfocitos T $CD4^+$ marcadamente disminuidos según la edad o ausencia de proliferación de linfocitos T frente a estimulación). Para el diagnóstico de IDVC deben excluirse otras causas secundarias de hipogammaglobulinemia, estableciéndose un diagnóstico después del cuarto año de vida (15).

Criterios Diagnósticos de la Inmunodeficiencia Variable Común.	
Al menos uno de los siguientes:	○ Mayor susceptibilidad a infecciones. ○ Manifestaciones Autoinmunes. ○ Enfermedad Granulomatosa. ○ Linfoproliferación policlonal inexplicable. ○ Historia familiar de deficiencia de anticuerpos.
Disminución marcada de IgG e IgA, con o sin disminución de IgM (medido al menos en dos ocasiones; <2SD de los niveles normales para su edad).	
Y al menos uno de los siguientes:	○ Baja producción de anticuerpos posterior a la vacunación (y/o ausencia de isohemaglutininas). ○ Estudio de fenotipo de linfocitos B con recuento disminuido de células B de memoria con cambio de isotipo (<70% del valor normal para la edad).
Deben excluirse otras causas secundarias de hipogammaglobulinemia (como infecciones, pérdida de proteínas, medicación, malignidad).	
Edad posterior al cuarto año de vida (aunque los síntomas pueden estar presentes desde una edad más temprana).	
No presentar evidencia de deficiencia profunda de células T definido por alguna de:	
	○ Recuento de linfocitos T $CD4^+$ marcadamente disminuidos según la edad (2-6 años <300 cel/ microL, 6-12 años <250 cel/microL, >12 años <200 cel/ microL). ○ Proliferación de células T ausente.

Tabla 1. Criterios Diagnósticos de la Inmunodeficiencia Variable Común (15).

Recientemente la Unión Internacional de Sociedades Inmunológicas (IUIS), publicó la actualización de la Clasificación Fenotípica de Errores Congénitos Humanos de la Inmunidad (16), donde clasifican a los fenotipos de IDVC dentro del grupo de patologías con deficiencia predominante de anticuerpos, asociado a hipogammaglobulinemia, con recuento de linfocitos B >1% **(Figura 1)**. Dentro de este grupo destaca IDVC: sin defecto genético específico, el síndrome de activación p110δ (APDS), haploinsuficiencia de IKAROS, así como deficiencias asociadas a PTEN, ARHGEF1, SH3KBP1, SEC61A1, RAC2, CD20, TACI, BAFF-R, TWEAK, IRF2BP2, CD19, CD81, CD21, TRNT1, NFKB1/2, ATP6AP1 y MOGS.

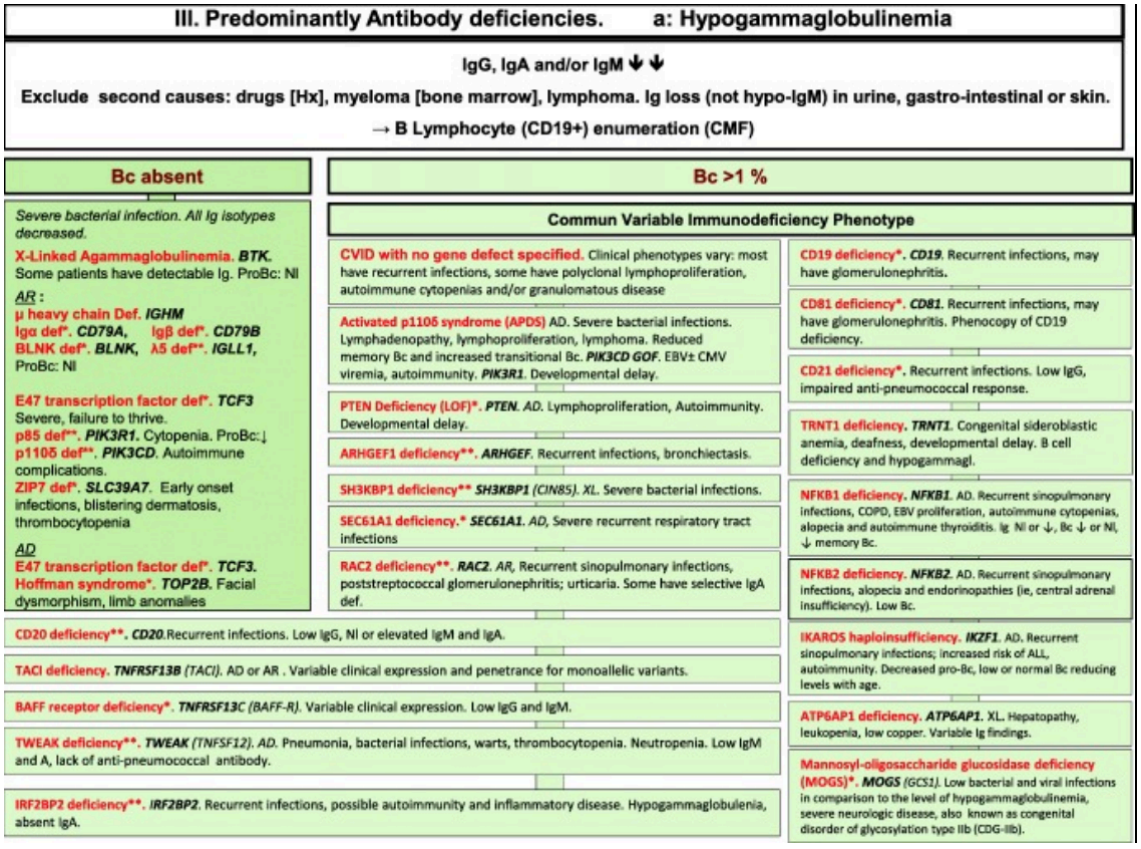


Figura 1. Clasificación Fenotípica de la Inmunodeficiencia Variable Común según IUIS (actualización de 2019), Figura extraída de Bousfiha A, Jeddane L, Picard C, et al. 2020 (16),

2.Epidemiología

2.1 Incidencia, Prevalencia y Distribución Etaria de la Enfermedad

Según diversas fuentes publicadas, la prevalencia geográfica de IDVC varía, siendo mayor en Norteamérica y Europa, con una incidencia que oscila entre 0,01 a 0,04 por 100.000

habitantes/año (17) y una prevalencia estimada de 1 en 25.000 a 50.000 individuos, siendo menor en Asia, Australia y África (18). Esto puede tener relación con la variabilidad genética, así como con la disponibilidad de métodos diagnósticos y registros poblacionales en cada región. En cuando a la distribución por sexo, encontramos que ambos sexos se ven igualmente afectados (19,20), sin una clara asociación entre sexo y población estudiada.

Aunque puede presentarse a cualquier edad, su debut clínico tiende a asociarse a dos picos etarios principalmente, uno en la infancia (5-10 años) y otro después de la pubertad, principalmente entre los 20 y 45 años (21–24). Diversos grupos de estudio han investigado si existe alguna diferencia entre la edad de debut y las manifestaciones/evolución de la enfermedad, destacando la otitis media y el retraso en el crecimiento y desarrollo las manifestaciones clínicas más observadas en la edad pediátrica, mientras que la bronquitis, artritis y astenia las manifestaciones más prevalentes en la edad adulta (21).

3.Etiología de la Inmunodeficiencia Variable Común

No se conoce un defecto único y específico como causante de IDVC. Hasta la fecha, las múltiples alteraciones que derivan de la enfermedad no se atribuyen, como en otras IDPs, a la modificación en un único gen. La IDVC parece ser el resultado de una variedad de factores que contribuyen a un defecto en la producción de anticuerpos, existiendo factores ambientales, epigenéticos y genéticos que pueden estar implicados (4,10,22,25,26).

3.1 Factores Ambientales y Epigenética

Li et al refieren que la causa predisponente de IDVC puede atribuirse hasta un 18% a herencia monogénica, mientras que el 82% restante es atribuible a factores externos (27), entre los que destacan (**Figura 2)** :

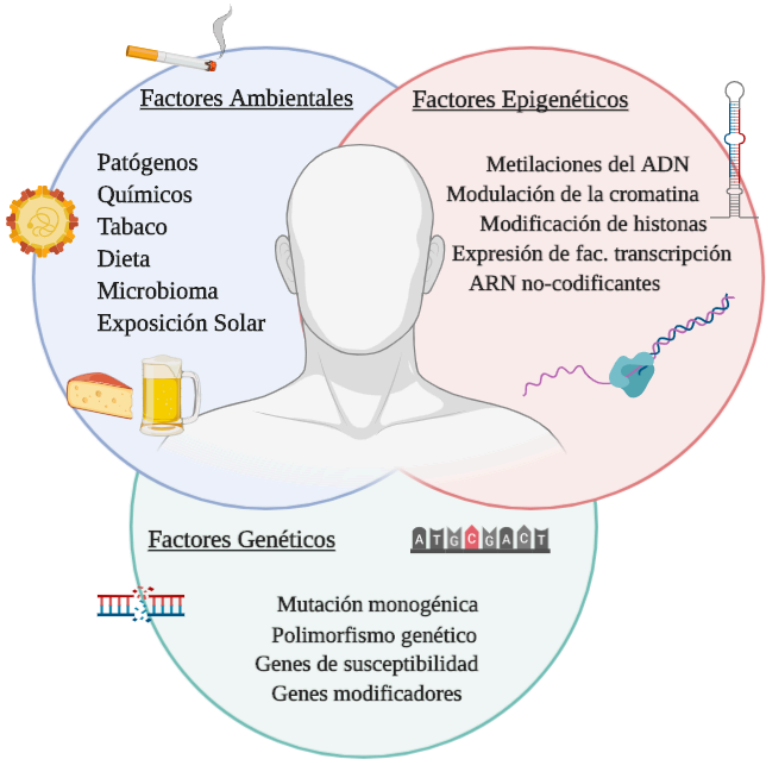


Figura 2. Factores Implicados en la Etiología y Fisiopatología de la Inmunodeficiencia Variable Común. Created with BioRender.com

El Microbioma

El microbioma intestinal modula el sistema inmunitario del huésped a través de sus componentes estructurales y metabolitos (28–30). Existe una clara sinergia entre ambos, tanto que varios investigadores han llegado a plantear que forman parte de un "metasistema" (31–33). Ejemplos de esta sinergia derivan del papel de productos bacterianos, como los lipopolisacáridos (LPS) en la activación de la cascada de respuesta innata, por medio del reconocimiento de patrones asociados a patógenos (PAMPS) (32), o en la generación de un estado pro-inflamatorio debido al desbalance en el sistema inmunitario asociado a la alteración del crecimiento microbiano a nivel intestinal (29).

En los pacientes IDVC, se han encontrado alteraciones que afectan el equilibrio de este metasistema (32,33). Por un lado, se ha descrito una disbiosis intestinal, con una marcada reducción de la diversidad alfa y diferencias en el perfil taxonómico en comparación con la población general, sin clara asociación al uso crónico de antibióticos (34). El perfil alterado del microbioma intestinal posiblemente podría modular la permeabilidad intestinal con la posterior elevación del LPS y la activación inmunológica crónica mediada por células T (33–35). Otros grupo de investigadores han descrito la asociación de taxones bacterianos potencialmente

relacionados con la enteropatía en IDVC (36), así como, el posible vinculo entre síntomas clínicos constitucionales y el sobrecrecimiento bacteriano a nivel intestinal (37,38), responsable de cuadros de diarrea crónica, con pérdida de peso severa y, sobre todo, malabsorción de nutrientes con déficit vitamínicos en estos pacientes (38).

Epigenética

El proceso de modificación de la expresión génica, influenciada por factores externos y no derivadas de una mutación en su línea germinal o en su secuencia, es lo que denominamos epigenética, capaz de inducir o inhibir señales asociadas a la respuesta inmunológica específica de cada individuo (39). Es un proceso activo, dinámico, acumulativo y potencialmente reversible (22), donde sus principales alteraciones se basan en metilaciones del ADN, modulación de la cromatina, modificación de histonas, expresión de factores de transcripción y ARN no-codificantes (22,40,41). Estos últimos desempeñan un papel fundamental en la adecuada interacción entre los linfocitos T y B (40).

El sistema inmunitario es especialmente susceptible a los cambios epigenéticos debido a la frecuente variabilidad tanto funcional como morfológica de las células inmunológicas durante su desarrollo, en especial durante el desarrollo de los linfocitos B (42). También presenta implicación en la respuesta inmunitaria innata asociada con señales intracelulares que inducen cambios metabólicos profundos y modificaciones epigenéticas que resultan en la reprogramación de las células de inmunidad innata, procesos denominado inmunidad entrenada, con potenciales aplicaciones en el contexto de la enfermedad (43,44). La implicación de la modulación de la cromatina y la modificación de histonas en la patogénesis de la IDVC se cree que está ligada a un defecto en la diferenciación y desarrollo del linfocito B, condicionando la disminución de las células vírgenes y de memoria con cambio de isotipo, con incremento de las $CD21^{low}$ (39).

En los diferentes estudios genéticos en sujetos con IDVC se han descrito un 15 a 20% de mutaciones monogénicas asociadas a la enfermedad, que presentan una fuerza de asociación causal relativamente baja para explicar en su totalidad el patrón de herencia de la IDVC (27,45,46), por lo que se considera que ésta pudiera estar determinada, bien por un perfil poligénico y/o bien asociada a fenómenos epigenéticos (22,47,48). Entre los genes con mayor riesgo de hipermetilación descritos en los pacientes con IDVC y IDVC-*like* destacan: PIK3CD, BCL2L1, RPS6KB2, TCF3, KCNN4 y PAX5 (22,49,50). La realización de estudios de exoma

completo en pacientes con IDVC, que permitan determinar variantes deletéreas implicadas en la regulación epigenética, podrían ser claves en el diagnóstico molecular de esta enfermedad, así como interesantes dianas terapéuticas basadas en medicina de precisión en la IDVC.

3.2 Factores Genéticos

En la mayoría de los casos, la IDVC ocurre de forma esporádica, la herencia familiar representa un 10% de los mismos (51). Se han descrito principalmente patrones autosómicos dominantes con penetrancia variable, autosómicos recesivos y ligados al cromosoma X (52). Se estima que alrededor del 20% de los pacientes con IDVC presentan antecedentes de déficit selectivo de IgA en un familiar de primer grado (53).

A diferencia de otras IDP, la IDVC puede presentar asociación tanto con mutaciones en un gen simple (MSH5, CD81 y CD20) (54–56), como asociada a polimorfismos (26,47). Se considera que el responsable de la diversidad de los fenotipos clínicos de la IDVC deriva de la multiplicidad de alteraciones genéticas expresadas en cada paciente (22). Entre los principales defectos genéticos intrínsecos descritos destacan: las alteraciones derivadas de la mutación en CD19 (16p11.2), mutaciones en ICOS (2q33)/CTLA-4 y alteraciones en TNFRSF13B y TNFRSF13C, entre otras (4,51,55,57).

Defectos monogénicos en varios miembros de la superfamilia de receptores del factor de necrosis tumoral (TNF) se han relacionado con la patogénesis de la IDVC, principalmente en: el gen activador transmembrana y modulador del calcio (TACI, codificado como TNFRSF13B), el factor activador de linfocitos B dependiente de la familia del receptor de factor de necrosis tumoral (BAFF-R codificado como TNFRSF13C), el inductor débil de apoptosis TNF-like (TWEAK, codificado como TNFRSF12) y el CD27 (codificado como TNFRSF7). Se conoce que BAFF-R y TACI participan en el desarrollo y activación de la célula B, asociados al ligando de inducción de proliferación (APRIL) y/o BAFF (58,59). Igualmente, TACI regula la función del receptor de la célula B (BCR), así como los receptores de tipo toll, que pueden a su vez condicionar el desarrollo de autoinmunidad (60). Según estudios previos, solo de un 8% a un 10% de los pacientes con IDVC presentan un defecto en TACI, encontrando una expresión similar en la población general, por lo que se desconoce cuál es su implicación patogénica real.

4.Fisiopatología de la Inmunodeficiencia Variable Común

4.1 El sistema inmunitario en la fisiopatología de la IDVC

A pesar de años de investigación sobre la IDVC, la patogenia exacta de esta enfermedad sigue siendo desconocida. La identificación de defectos monogénicos y las modificaciones epigenéticas han incrementado la comprensión de esta compleja enfermedad (23,50,61,62). Su variabilidad clínica sugiere un defecto inmunoregulatorio múltiple, que resulta en una vía final común de hipogammaglobulinemia y desregulación inmunológica (23,24,63). Varios estudios han descrito numerosas alteraciones en el sistema inmunitario, tanto innato como adaptativo, siendo su perfil más común, la alteración en la formación de anticuerpos (10,21). Existe un desequilibrio entre la respuesta humoral y celular mediada por linfocitos en la IDVC, expresado como un defecto en la capacidad del linfocito T de estimular al linfocito B (19,20), y/o en la respuesta de éste frente al estímulo antigénico (11,12).

Alteraciones en el linfocito B:

El desarrollo ontogénico, la maduración y la diferenciación del linfocito B se produce según estadios secuenciales, iniciándose su estadio más precoz en la medula ósea, pasando por estadios transicionales dentro de la zona marginal del ganglio linfático y completando su maduración en compartimientos periféricos (**Figura 3)** (64–66). A pesar de que hasta un 90% de los pacientes con IDVC presentan un recuento absoluto de linfocitos B dentro de la normalidad (64), al evaluar el fenotipo de memoria B, se pueden detectar alteraciones en los diferentes estadios de diferenciación, predominando un bloqueo en estadios iniciales, lo cual condiciona parte del papel efector y funcional del linfocito B, especialmente en su función de producción de anticuerpos.

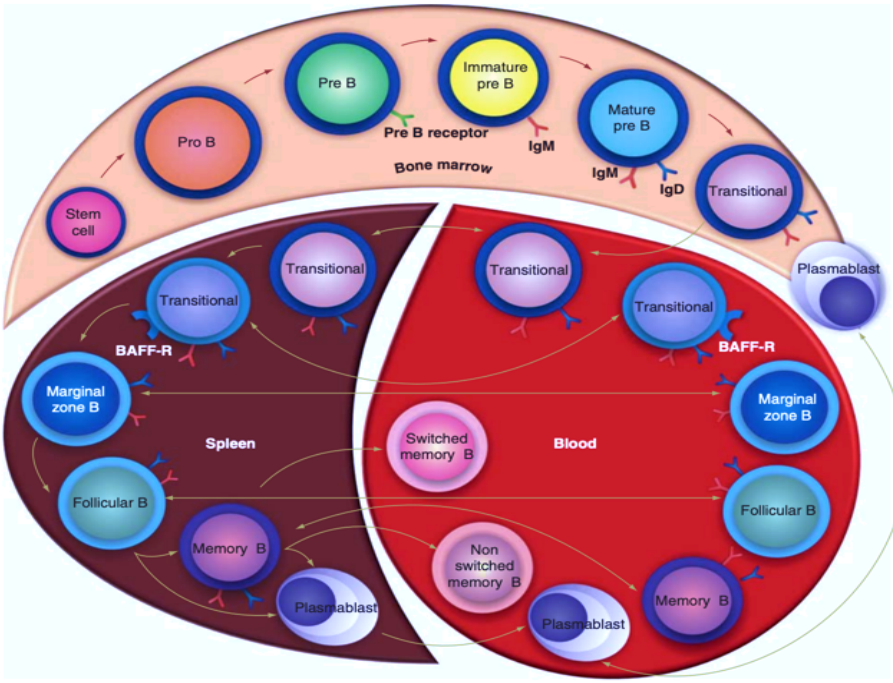


Figura 3. Desarrollo y Maduración normal del Linfocito B. Extraído de Kumar y Bhatia, 2013 (66).

Una proporción de los pacientes con IDVC que oscila entre 80% y 90% (66) presenta disminución en los linfocitos B de memoria con cambio de isotipo, así como de subsiguiente producción de inmunoglobulinas (Ig) por las células plasmáticas, siendo este proceso crítico en la respuesta de producción de anticuerpos específicos de antígeno, que contribuye esencialmente a la presentación clínica de la enfermedad (64).

Las alteraciones descritas en el linfocito B durante su maduración se han asociado con ciertas manifestaciones clínicas propias de la IDVC (13,14,64,67), como esplenomegalia, proliferación linfocítica, enfermedad granulomatosa, enfermedad pulmonar crónica (bronquiectasias), así como citopenias autoinmunes (5,64,67–69). En ciertos pacientes estos porcentajes pueden variar en el tiempo, por lo que se recomienda que estas variables inmunológicas sean evaluadas en el tiempo durante el seguimiento del paciente con IDVC.

Alteraciones en el linfocito T:

A pesar que los criterios diagnóstico actuales para IDVC excluyen los pacientes con defectos severos en los linfocitos T (56,70), alteraciones en proporción, funcionalidad y señalización de los linfocitos T han sido descritos en los pacientes con IDVC, encontrando disminución de los linfocitos T totales y células T-CD4^{+} vírgenes, así como alteración en la respuesta de las células

T reguladoras (T_{Reg}), y de la proliferación frente a mitógenos y antígenos específicos (19,63), expansión de linfocitos T gamma/delta (71), alteración en la señal de transducción en el receptor de la célula T (TCR) y alteraciones en diferentes vías de señalización celular, como la vía asociada a los receptores de tipo toll (TLR) (72–74). Se piensa que la mayoría de estas alteraciones se deben a una desregulación en la selección tímica de los T_{Reg}, consecuencia de un aumento en la activación de los linfocitos T $CD4^+$ autorreactivos, lo que induciría activación acelerada las señales de apoptosis, generando una reducción en el recuento total de las células T $CD4^+$ (19,33,63). La disminución de T_{Reg} se ha asociado con manifestaciones clínicas como autoinmunidad (20,75), desarrollo de granulomas (76) y esplenomegalia (77).

Existe controversia sobre el papel que desempeñan los linfocitos T *helper* (Th) en la fisiopatología de la IDVC (63). Ciertas citoquinas características de diferenciación Th2, como la IL-4 e IL-10 se han encontrado elevadas en estos pacientes, lo que no sucede con las citoquinas Th1 (48,78). En cuanto a las citoquinas asociadas a la vía Th17 (IL-17A , IL-17F, IL-22 y IL-21), hay autores que reportan una disminución de su activación en pacientes con IDVC (79). La diferenciación Th17 contribuye a la fisiopatología de enfermedades inflamatorias y autoinmunes, así como en la defensa frente a infecciones extracelulares de bacterias y hongos (78,80).

Respecto a los linfocitos T citotóxicos, se ha observado una reducción de linfocitos T $CD8^+$ vírgenes y memoria efectores, con mayor expresión de HLA-DR^+, $CD38^+$ y $CD38^+$HLA-DR^+ en un subgrupo de pacientes con IDVC (63,81,82). Este perfil de activación se ha relacionado con autoinmunidad, esplenomegalia, proliferación linfoide y enfermedad granulomatosa (81).

Otras alteraciones:

Alteraciones en otras poblaciones celulares han sido descritas en estos pacientes (83). Las células dendríticas (CDs) son células profesionales presentadoras de antígeno (CAP) implicadas en la inducción de la respuesta T, así como en la diferenciación de los linfocitos B a células plasmáticas (84,85). Estudios en pacientes con IDVC, han mostrado una disminución tanto en número como en capacidad funcional de las CDs, sobre todo plasmacitoides, que son las principales productoras de interferón de tipo I (IFN I), con alteración en la expresión de moléculas co-estimuladoras como CD80, CD86, HLA-DR e IL-12 (84). Se piensa que las alteraciones funcionales de las CDs, podrían estar implicadas en el defecto se reconocimiento

de antígeno por parte de las células T $CD4^+$ y por consiguiente la señal de activación del linfocito B para la producción de anticuerpos específicos (86).

Se han descrito alteraciones a nivel de los monocitos en pacientes con IDVC (**Figura 4**), que pueden resultar en el desarrollo de ciertas manifestaciones clínicas asociadas a la enfermedad, como la malignidad, procesos linfoproliferativos, alteraciones autoinmunes y enfermedad pulmonar derivadas de un perfil proinflamatorio crónico (87). Esta sobreexpresión de moléculas de activación en monocitos/macrófagos podría favorecer aún más la balanza hacia una respuesta predominantemente Th2 frente a Th1/Th17, descrita previamente (88–90).

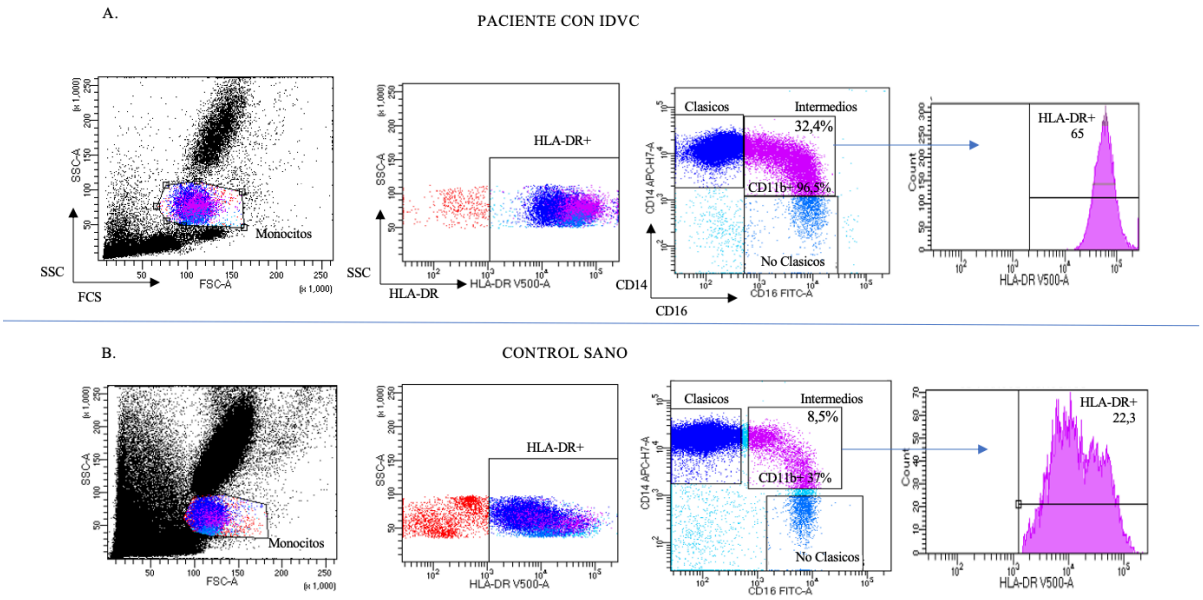


Figura 4. Estrategia de gateo para la caracterización fenotípica de los monocitos circulantes por citometría de flujo multiparámetrica en (**A**) un paciente con IDVC versus (**B**) un control sano. Se puede observar un aumento de la subpoblación de monocitos intermedios en el paciente con IDVC en comparación con el control sano.

Las células NK, población celular de la inmunidad innata con un papel predominante frente a infecciones víricas y vigilancia anti-tumoral (12,91,92), se encuentran significativamente disminuidas en pacientes con IDVC (93) (**Figura 5)**, hallazgo que se ha relacionado con un mayor riesgo de infecciones víricas (además del defecto mencionado del IFN I), bacterianas invasivas y con una mayor predisposición a desarrollar complicaciones no infecciosas (94).

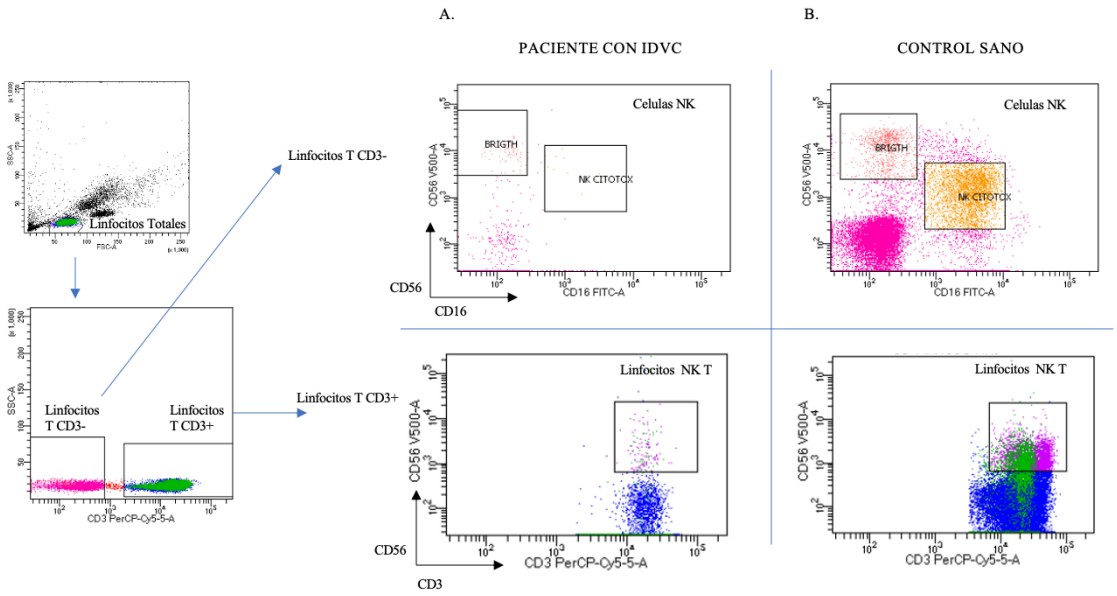


Figura 5. Estrategia de gateo para la caracterización fenotípica de las células NK por citometría de flujo multiparamétrica en (**A**) Paciente con IDVC versus (**B**) Control Sano. Se puede observar la disminución global de células NK y NK T en el paciente con IDVC en comparación con el control sano.

5.Curso Clínico de la Inmunodeficiencia Variable Común (Figura 6).

5.1 Manifestaciones infecciosas

Las infecciones son la manifestación clínica más frecuente de los pacientes con IDVC, destacando principalmente las infecciones respiratorias, gastrointestinales y cutáneas (86,95–97). Las infecciones respiratorias de vías altas y bajas recurrentes ocupan el primer lugar; pudiendo generar un daño pulmonar establecido, frecuentemente como bronquiectasias y derivar así en una enfermedad pulmonar obstructiva crónica (98–100). De la misma forma, diversas afecciones pulmonares como la enfermedad intersticial difusa podría condicionar la aparición de enfermedad pulmonar restrictiva, con implicación directa en la morbimortalidad de la enfermedad (98). Las bronquiectasias y la enfermedad pulmonar intersticial son dos de las complicaciones más frecuentes, mientras que la inflamación crónica de los senos paranasales y la otitis media recurrentes pueden derivar en hiposmia, anosmia y/o disminución de la capacidad auditiva (sordera) progresiva (98,101,102), hallazgos difíciles de identificar si no se realiza una adecuada anamnesis y valoración clínica de los pacientes. Entre los principales microrganismos asociados a infecciones descritos en pacientes con IDVC se encuentran: *Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis y Staphilococcus aureus,* así como microorganismos atípicos como *Pneumocystis jirovecii y Mycoplasma pneumoniae* (103,104).

En cuanto a las infecciones gastrointestinales (GI) en la IDVC, se ha descrito cierta predisposición en aquellos pacientes que presentan niveles marcadamente disminuidos de IgA (105). Entre las manifestaciones clínicas, son frecuentes la diarrea crónica persistente y cuadros malabsortivos asociados a agentes como *Giardias sp, Clostridium difficile, citomegalovirus* (CMV), *Helicobacter pylori* y *Norovirus tipo II,* entre otros (96,97). Es importante resaltar que aunque la prevalencia de la infección por *Helicobacter pylori* no sea significativamente mayor en relación con la población general, sí se observa un aumento en la susceptibilidad de desarrollo de malignidad derivada de la infección en pacientes con IDVC (106,107). Al existir un protocolo de erradicación de la misma, se ha propuesto la obligatoriedad de tratarlo en estos pacientes (38,105).

Ciertas afecciones cutáneas descritas en la IDVC pueden ser reflejo de procesos secundarios asociados a la enfermedad, como las infecciones bacterianas de la piel (impétigo, celulitis y forúnculos) (4,68,97).

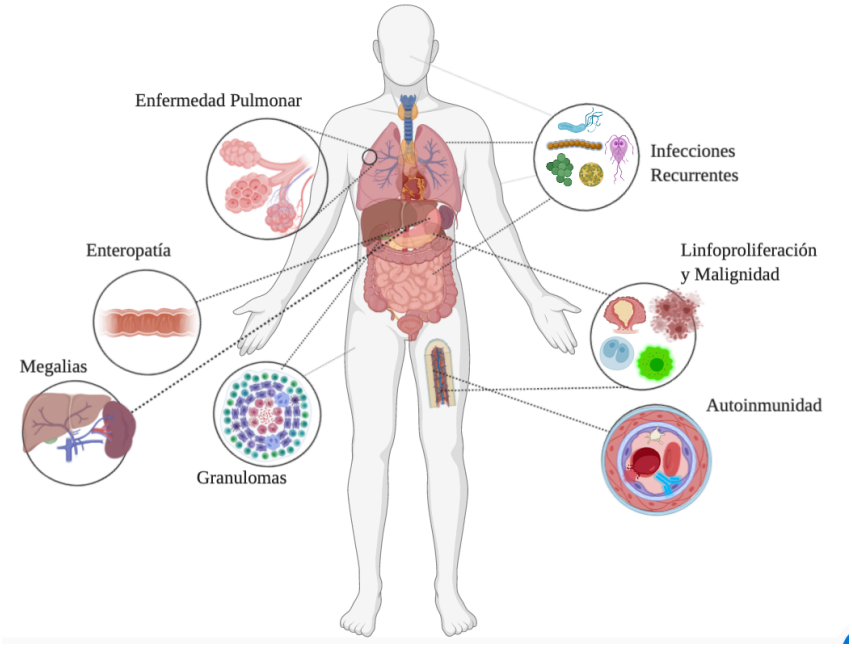


Figura 6. Principales Manifestaciones Clínicas de la Inmunodeficiencia Variable Común. Created with BioRender.com

5.2 Manifestaciones no infecciosas

Enteropatía: Un subgrupo de pacientes con IDVC presentan diversos trastornos GI no asociados a infecciones, destacando la enfermedad inflamatoria intestinal, que se presenta

frecuentemente como colitis inflamatoria, enteropatía pierde proteínas, gastropatía celiaca-*like* y enfermedad hepática, etc. (108). La presentación clínica de estos trastornos se asocia con diarrea crónica refractaria a tratamiento, habitualmente con pérdida de peso y malnutrición (108–110). El hallazgo más característico en los estudios histopatológicos es la hiperplasia folicular linfoide presenta en gran número de los pacientes con manifestaciones clínicas GI. El factor predisponente de este hallazgo no se conoce bien (105,111,112). De la misma forma, los pacientes con IDVC pueden presentar afectación hepática con aumento de fosfatasa alcalina, sin evidencia de infección vírica. La biopsia hepática muestra infiltración de linfocitos periportales y colestasis leve a moderada (86).

Enfermedad Pulmonar: Las principales manifestaciones asociadas a la esfera pulmonar en IDVC son la enfermedad pulmonar crónica, como bronquiectasia no infecciosa, enfermedad pulmonar intersticial, inflamación granulomatosa y enfermedad pulmonar obstructiva / restrictiva, entre otras (98,100,103). Estas manifestaciones pueden ser consecuencia de infecciones respiratorias recurrentes o bien presentarse de forma aislada derivadas de un proceso proinflamatorio o asociadas a linfoproliferación.

Autoinmunidad: las enfermedades autoinmunes tienen una prevalencia estimada del 10-30% (113,114). Los desórdenes hematológicos son los más comúnmente relacionados con la autoinmunidad, con una prevalencia alrededor del 20%. Las citopenias periféricas no solo son la manifestación más frecuente, sino que también se han visto asociadas al pronóstico de la enfermedad (2,5). Las trombocitopenias autoinmunes hasta en un 12% de los pacientes según las series (115,116). Es importante señalar que alrededor del 60% de los pacientes con IDVC, el diagnóstico de citopenia puede preceder por muchos años a la detección de hipogammaglobulinemia (117,118). Entre las citopenias, la más frecuente es la trombocitopenia inmunológica, seguida de la anemia hemolítica autoinmune y el síndrome de Evans (bicitopenia) (2,101). Otras expresiones clínicas menos frecuentes asociadas a autoinmunidad en pacientes con IDVC son la artritis reumatoide, lupus eritematoso sistémico (LES), el síndrome de Sjögren, la tiroiditis autoinmune y el vitíligo (2,33,86,115).

A pesar de la clínica de autoinmunidad, un gran porcentaje de pacientes presentan negatividad en los tests de autoanticuerpos, lo que podría estar en relación con la alteración de la señalización implicada en la producción de anticuerpos específicos presentes en estos pacientes

o a bien a una respuesta aberrante frente a autoantígenos por parte de las T_{Reg} (2,20,67). Se requiere evidencia sólida sobre los mecanismos exactos del desarrollo de autoinmunidad en pacientes con IDVC.

Manifestaciones dermatológicas: las manifestaciones dermatológicas asociadas a la IDVC son muy diversas. En general, las principales descritas son la alopecia areata, alopecia universalis, dermatitis atópica, vasculitis cutáneas, incluyendo la poliarteritis nodosa, etc. (10,101,115). Igualmente, se pueden observar lesiones cutáneas asociadas a manifestaciones autoinmunes, como vitíligo, psoriasis o LES; enfermedad autoinflamatoria como pioderma gangrenoso; granulomas necrotizantes y no necrotizantes con presencia de pápulas, placas, nódulos y úlceras; erupción maculopapular; púrpura trombocitopénica y granulomas (86,113,119).

Linfoproliferación: la infiltración linfocítica policlonal en pacientes con IDVC incluye: la linfoproliferación policlonal, linfadenopatía persistente inexplicada, neumonitis intersticial linfoide (NIL) y granuloma no infeccioso (5). La prevalencia de la enfermedad granulomatosa puede variar según la población estudiada (114,120), observándose en el 8% -22% de los pacientes con IDVC según las series publicadas (95,120). Esta patología podría estar asociada con una mayor incidencia de autoinmunidad y una supervivencia más corta (121). En la mayoría de los casos, las lesiones granulomatosas se encuentran en el pulmón, los ganglios linfáticos, el hígado y la piel (95,120), pueden presentarse en varios órganos simultáneamente con presentaciones clínicas muy variadas que sugieren una forma inusual de reacción inflamatoria (122). La infiltración linfocítica policlonal se asocia con un riesgo 8 veces mayor para las mujeres y un riesgo 10 veces mayor para los hombres de malignidad linfoide, según estudios publicados (114,123).

Malignidad: se ha asociado la edad del paciente, el estímulo infeccioso crónico y la disbiosis, como factores externos que, junto con los factores oncogénicos intrínsecos de la IDVC influyen como mecanismos fisiopatológicos complejos asociados al riesgo de desarrollar malignidad (124). El cáncer es 1,4 a 5 veces mayor en las IDP con respecto a la población general (125,126), de los cuales el 70% corresponde a neoplasia linfoide (127,128). Las personas con IDVC tienen un riesgo 5 a 10 veces mayor de desarrollar neoplasias hematológicas (125,126,129), mientras que se ha descrito una incidencia inesperadamente menor en la

mayoría de los cánceres comunes que la población general (130,131). El cáncer es una de las principales causas de mortalidad en la IDVC, después de las complicaciones derivadas de las infecciones recurrentes (132,133). Por tanto, el diagnóstico precoz y el tratamiento de la neoplasia maligna es una prioridad. Se estima que esta incidencia aumenta de forma directa con el debut más tardío de la enfermedad (125,126,134).

Entre las principales malignidades hematológicas, encontramos las derivadas de una linfoproliferacion policlonal inicial, siendo los linfomas, especialmente el tipo no Hodgkin de linfocitos B (principalmente extranodal) el descrito con mayor prevalencia (101). El siguiente en frecuencia es el asociado a las malignidades GI, que se describe con un riesgo elevado de hasta 10 veces mayor de desarrollo de cáncer gástrico en pacientes con IDVC en relación a la población normal (135). Patógenos estrechamente relacionados con el cáncer gástrico son el virus de Epstein-Bar y el *Helicobacter pylori* (37,111,112).

6.Clasificación de la Inmunodeficiencia Variable Común

6.1 Identificación de Biomarcadores Diagnósticos y Pronósticos

Se han propuesto en los últimos años diversos biomarcadores predictivos, como los niveles de inmunoglobulinas séricas, los niveles de sFLC, el fenotipo de linaje T, entre otros, asociados a diversas manifestaciones autoinmunes, formas granulomatosas, riesgo de infecciones graves o desarrollo de hiperplasia linfoide (82,86,120,127,136).

6.2 Identificación de Fenotipos Clínicos en la IDVC

Chapel et al. propusieron en 2008 una clasificación basada en fenotipos clínicos de la IDVC (114), que posteriormente fue revisada, publicando la revisión de los criterios en 2012 (5). Con esta clasificación se consigue englobar aproximadamente el 80% de los pacientes con IDVC dentro de solo uno de los cuatros fenotipos clínicos principales, que comprenden: la citopenia; infiltración linfocítica policlonal; enteropatía inexplicada; y complicaciones no relacionadas con la enfermedad (pacientes que presentan clínica de infecciones exclusivamente) (5).

6.3 Identificación del Perfil Genético

El análisis genético de un panel de genes relacionados con IDVC se considera esencial en el enfoque diagnóstico diferencial y de medicina de precisión (42,55,124,137,138). Al menos en los pacientes con IDVC con fenotipo grave, se debe considerar la secuenciación genética para detectar la respuesta responsable de la presentación clínica (26,55,139).

Varios estudios han asociado la expresión alélica de HLA-DR y HLA-DQ con el riesgo relativo de presentar enfermedades autoinmunes, como la enfermedad de Addison, la enfermedad celíaca, la tiroiditis autoinmune, la diabetes mellitus tipo I, la miastenia gravis, artritis reumatoide y el LES (1,140,141).

6.4 Biomarcadores de Inicio de Tratamiento

En 2009, el Comité de Expertos de Inmunodeficiencia Primaria de la Unión Internacional de Sociedades Inmunológicas (IUIS) redefinió la IDVC como un "síndrome", debido a la amplia variabilidad de hallazgos clínicos y de laboratorio que comprende, catalogándola con el término de "trastornos de inmunodeficiencia variable común" (54). Más recientemente, expertos internacionales en IDVC entre los que destaca Charlotte Cunningham-Rundles, propusieron que el diagnóstico de IDVC debería basarse en primer lugar en criterios de laboratorio, nuevos biomarcadores y en el fenotipo genético (142).

Las pautas actuales apoyan el IgRT en pacientes con IDVC sintomática como el pilar de la prevención contra las infecciones por patógenos (10,143–145). La puntuación para IgRT propuesta por Charlotte Cunningham-Rundles (143), se basa en la asignación de una escala según los datos obtenidos de pruebas analíticas de laboratorio que incluyen niveles de inmunoglobulina (IgG, IgA e IgM) y respuestas específicas de anticuerpos a antígenos proteicos y polisacáridos. Así como, medidas clínicas, como infección bacteriana grave (meningitis, sepsis y osteomielitis), hospitalizaciones, neumonías e infecciones del tracto respiratorio superior y uso de antibióticos. Otras características valoradas comprenden síntomas gastrointestinales, como diarrea infecciosa, pérdida de peso, retraso del crecimiento, malabsorción, gastroenteritis crónica, enfermedad inflamatoria intestinal e historial de enfermedad autoinmune (comúnmente trombocitopenia autoinmune o anemia hemolítica autoinmune). Entre los hallazgos físicos, tomados en cuenta en este sistema de puntuación se encuentra la linfadenopatía, esplenomegalia, antecedentes de esplenectomía y evidencia de

enfermedad pulmonar, esta última debe ser demostrada por pruebas función pulmonar alterada o evidencia de bronquiectasia en tomografía computarizada de tórax.

Las recomendaciones para el inicio de terapia con inmunoglobulina se deben considerar en aquellos pacientes que la sumatoria de los ítems de sus datos de laboratorio presenten puntajes igual o mayor de 10 puntos. En los casos en que la puntuación de laboratorio sea inferior a 10, otras consideraciones, incluidas las puntuaciones clínicas, pueden ser tomadas en cuenta, considerándose la instauración de la terapia con inmunoglobulina en pacientes con puntajes acumulativos superiores a 16 puntos. Para los pacientes con puntajes acumulativos de 10 a 16, la decisión debe ser tomada según criterio y experiencia medica. Un puntaje bajo no descarta la posibilidad de requerir terapia de inmunoglobulina en el futuro.

7.Diagnóstico de la Inmunodeficiencia Variable Común

Los pacientes con IDVC pueden presentar un perfil de manifestaciones clínicas y/o alteraciones inmunológicas asociadas muy amplio y heterogéneo, lo cual dificulta y retrasa el diagnóstico, condicionando así el pronóstico del paciente. Se ha descrito que los miembros de una misma familia con idéntica mutación pueden presentar un debut y manifestaciones clínicas muy diversas de la enfermedad (55,146). Una alta sospecha clínica y un seguimiento adecuado constituyen el pilar fundamental para reducir la morbimortalidad en la IDVC (26,101,147–149).

En general, el examen físico de los pacientes con IDVC puede ser normal. Deben buscarse específicamente síntomas y signos asociados a dolencias crónicas que no deben ser pasadas por alto. Entre los principales aspectos a evaluar destacan: la congestión nasal derivada de cuadros de sinusitis crónicos, cicatrices timpánicas asociadas a otitis recurrentes, síntomas B (pérdida de peso, sudoración y fiebre nocturna) asociados a una posible malignidad linfoide, acropaquias, tos productiva crónica y disnea, derivadas de una enfermedad pulmonar crónica de base, al igual que hallazgos como linfadenopatías, esplenomegalia, artritis, conjuntivitis, muy frecuentes en esta patología (10,10,144,150). Todos estos aspectos deben evaluarse al diagnóstico y de forma rutinaria durante el seguimiento médico.

7.1 Laboratorio y Pruebas Paraclínicos al momento del diagnóstico

Además de una anamnesis detallada por órganos y aparatos y un examen físico completo, en todo paciente con sospecha de IDVC de deben realizar diversos test paraclínicos que permitan establecer el diagnóstico preciso, así como la situación y factores pronósticos del mismo. Como primera evaluación, se debe realizar un hemograma completo con distribución de la formula diferencial de leucocitos, frotis sanguíneo, test de Coombs en caso de anemia de reciente aparición **(Tabla 2).** Todo esto, brindará información importante, clara y útil en la evaluación inicial de la enfermedad.

Evaluación de Laboratorio y Test Paraclínicos para el diagnóstico y seguimiento de IDVC.	
Evaluación Inicial	○ Hemograma completo con formula diferencial leucocitaria ○ Bioquímica sanguínea ○ Test de Coombs (en caso de anemia de reciente aparición) ○ Sistemático de orina ○ Cuantificación de inmunoglobulinas séricas (IgG, IgA, IgM, IgE). ○ Determinación de producción de anticuerpos proteicos y polisacáridos post-inmunización. ○ Determinación de isohemaglutininas. ○ Estudio de los factores del complemento.
Evaluación Avanzada	○ Análisis de subpoblaciones linfocitarias (linfocitos T $CD3^+$, $CD4^+$, $CD8^+$, B $CD19^+$, y células NK $CD56^+$. ○ Estudio de fenotipo de memoria B. ○ Estudio fenotípico de linaje T (en caso de sospecha de alteración del perfil celular). ○ Análisis genético: NGS o exoma.
Otras evaluaciones	○ Pruebas de función pulmonar. ○ Pruebas de imagen dirigidas (descartar daño estructural, megalias, linfoproliferación y malignidad)

Tabla 2. Evaluación de Laboratorio y Test Paraclínicos para el diagnóstico y seguimiento de Inmunodeficiencia Variable Común (4,142,144,150,151).

Las principales pruebas inmunológicas basales frente a una alta sospecha de IDVC son:

Niveles de inmunoglobulinas séricas: Según guías de expertos la disminución de IgG asociados a la disminución de IgA o IgM por debajo de 2 DE constituye un dato clave para el diagnóstico de IDVC (8). La IgE también debe cuantificarse (152,153). Los niveles séricos de Ig varían según la edad del paciente y por consiguiente deben valorarse en ese contexto y conjuntamente con los niveles de proteínas totales o albúmina en sangre, descartando la asociación de patología por pérdida de proteínas a nivel intestinal o renal.

Siempre se deben descartar otras posibles causas de hipogammaglobulinemia. Entre las causas primarias, se debe valorar deficiencia de IgG1 e IgG2, síndrome hiper-IgM (HIGM), e inmunodeficiencias combinadas. Entre las causas secundarias se debe descartar el uso de fármacos, desórdenes asociados a supresión de la medula ósea, síndrome de *Goodpasture*, malignidades, enteropatías pierde proteínas, síndrome nefrótico, parasitosis, etc.

Determinación de producción de anticuerpos IgG específicos, ya sean naturales o por medio de la respuesta a vacunación:

En cuanto a la determinación de anticuerpos IgG específicos naturales, gran número de los pacientes con IDVC que desarrollan la enfermedad en edad adulta presentan IgG frente a los virus exantemáticos comunes de la infancia, lo que podría reflejar la competencia de las respuestas de anticuerpos en el momento de la interacción con estos patógenos (durante la infancia) y el mantenimiento de la memoria inmunológica a los mismos (119).

Para establecer el diagnóstico se requiere demostrar un defecto en la producción de anticuerpos específicos. De forma general se evalúa comparando los niveles basales de anticuerpos frente a antígenos proteico (por ejemplo, tras vacunación antitetánica o antidiftérica, Td) y antígeno polisacárido (por ejemplo, vacuna 23-valente antineumocócica o frente *Salmonella typhi*) con los niveles post-inmunización. El aumento normal debe ser al menos 4 veces superior al título basal frente a antígeno proteico y/ó al menos 3 veces superior para antígeno polisacárido, para la interpretación del defecto en la producción de anticuerpos específicos. En el caso de que la medición se realice por medio de serotipos individuales frente al polisacárido neumocócico, se define el déficit de producción como la imposibilidad de alcanzar valores de 1 en al menos el 50% de los serotipos estudiados. Está técnica no está disponible en España de forma rutinaria.

La prueba considerada *gold-standard* actual para evaluar la respuesta humoral anti-polisacárida T-independiente es la determinación de anticuerpos a la vacuna antineumocócica 23-valente (PPV) (154). Sin embargo, la inclusión de la vacuna adyuvante antineumocócica (13-valente) en el calendario vacunal infantil español, así como dentro de las indicaciones de vacunación de grupos de riesgo según la CDC, sumado a las limitaciones para la medición rutinaria de serotipos específicos frente a la vacuna, hace que la interpretación de estos frente a polisacárido puro sea más complicada (155–157). La respuesta a la inmunización puede estar influenciada además por la inmunidad del rebaño debido a la inmunización generalizada en la infancia y a

la exposición endémica a neumococo (158,159). Se ha propuesto la valoración conjunta con otra vacuna de antígeno polisacárido puro de la *Salmonella typhi* (Typhim Vi) como una herramienta accesoria útil para determinar la producción de anticuerpos específicos en esta población (155,159,160). Para la determinación de anticuerpos proteicos se emplea de forma general vacunas como la antitetánica, antidiftérica o frente a la rabia (3,10,150). La determinación de producción de anticuerpos debe realizarse de forma general previo inicio de tratamiento sustitutivo con gammaglobulinas y debe interpretarse en relación con el nivel basal de anticuerpos.

7.2 Como evaluación especifica de la IDVC, una vez establecido el diagnóstico es necesario determinar:

<u>Perfil inmunológico humoral y celular:</u>

- Análisis de subpoblaciones linfocitarias mediante citometría de flujo, principalmente linfocitos T $CD3^+$, $CD4^+$, $CD8^+$, B $CD19^+$ y células NK $CD56^+$.
- Fenotipo de memoria B en sangre periférica. Los linfocitos B se pueden clasificar según sus marcadores de superficie en los diversos estadios de desarrollo: células B vírgenes o naïve ($CD27^-IgD^+$), de memoria (IgD^+CD27^+), de memoria con cambio de isotipo ($CD27^+IgD^-$), células B $CD21^{low}$, de transición ($IgM^{++}CD38^+$), y plasmablastos ($CD38^{+++}IgM^-$).

El hallazgo más consistente en pacientes con IDVC es una disminución en la población de linfocitos B de memoria (12–14,64,88). Igualmente se describe una asociación del aumento significativo en las linfocitos B $CD21^{low}$ (grupo smB-21^{low}, según la clasificación de la EUROClass (12), con mayor riesgo de desarrollar esplenomegalia y/o enfermedad granulomatosa (12,161), de linfadenopatía en pacientes que presentan un aumento en las linfocitos B transicionales acompañado de una reducción severa en las linfocitos B de memoria con cambio de isotipo (14,161,162). Si bien el fenotipo de linfocitos B en sí mismo no es diagnóstico de IDVC, puede ayudar a determinar la gravedad de enfermedad y la posibilidad de progresión hacia complicaciones como la enfermedad granulomatosa, la esplenomegalia y la enfermedad linfoproliferativa, lo que permite el manejo o seguimiento de la enfermedad **(Figura 7).**

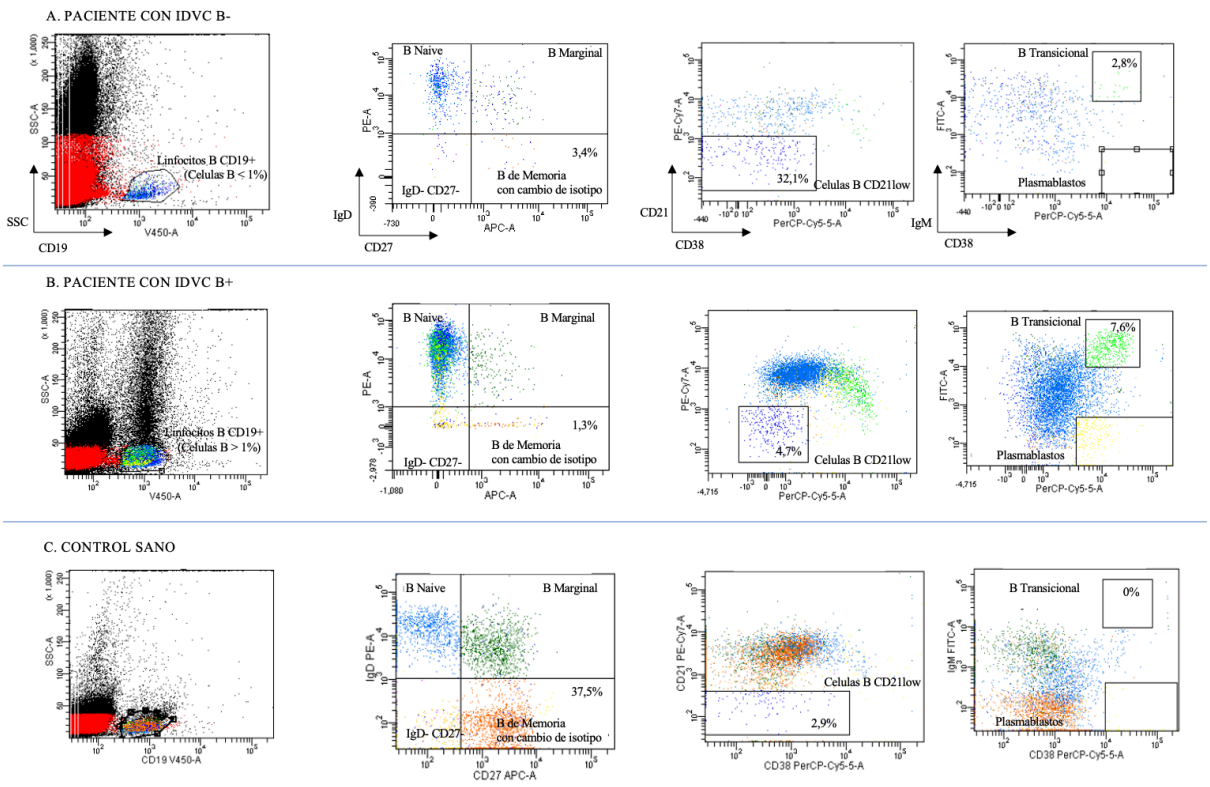


Figura 7. Estrategia de gateo para la caracterización fenotípica de los linfocitos B circulantes por citometría de flujo multiparámetrica en (**A**) un paciente con IDVC con recuento de linfocitos B <1%, grupo B- según EUROClass, versus (**B**) un paciente con IDVC con recuento de linfocitos B >1%, grupo B+ smB-21^{norm} según EUROClass. Se puede observar un mayor numero de las distintas subpoblación de linfocitos B en el paciente con IDVC **B** en comparación con el paciente con IDVC **A**.

-Estudio fenotípico de linaje T: Como se mencionó anteriormente, la presencia de defecto severo T es un criterio de exclusión para el diagnóstico de IDVC (56,70), siendo estos pacientes clasificados por ciertos autores como inmunodeficiencia combinada de inicio tardío "*Late onset combined immunodeficiency*" (LOCID) (56,70). Sin embargo, un subgrupo de pacientes con IDVC presentan alteraciones diversas en proporción, funcionalidad y señalización de los linfocitos T como defecto asociado a su inmunodeficiencia (19,76,81,82). Los más frecuentes consisten en linfocitopenia T $CD4^+$, disminución de la proliferación de linfocitos en respuesta a mitógenos y antígenos específicos, señalización defectuosa de los linfocitos T con disminución de los recuentos de T_{Reg}, niveles elevados de marcadores de activación de linfocitos T y alteraciones en la producción de citoquinas (163–166). Estas alteraciones se han visto relacionadas a un fenotipo más grave de la enfermedad comúnmente asociadas con enteropatía, esplenomegalia, granuloma y linfoma (167,168). En pacientes con

hallazgos sugerentes de alteración de la inmunidad celular, el perfil fenotípico de linaje T se evalúa mediante citometría de flujo (**Figura 8)** (169).

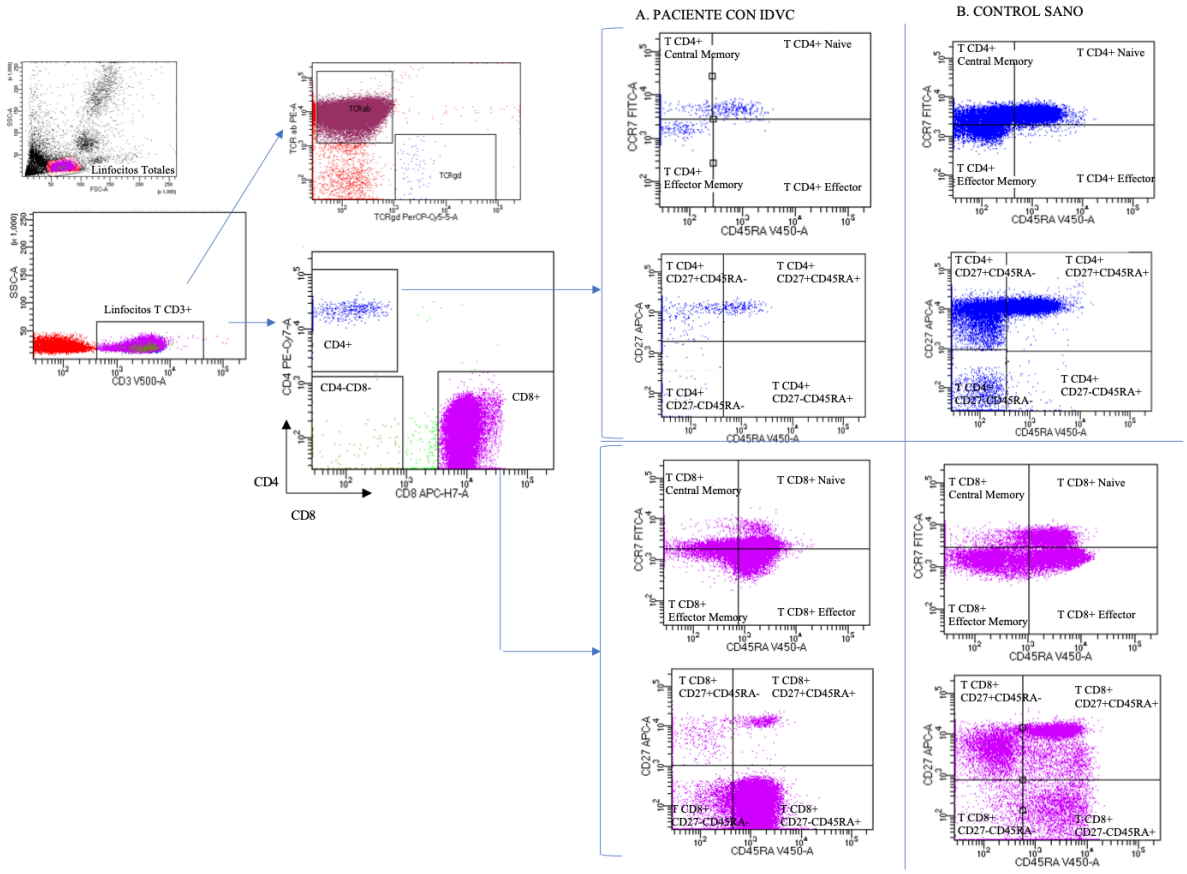


Figura 8. Estrategia de gateo para la caracterización fenotípica de los linfocitos T circulantes por citometría de flujo multiparámetrica en (**A**) un paciente con IDVC versus (**B**) un control sano. Se puede observar una disminución global de las diferentes subpoblaciones de linfocitos T $CD4^+$ y $CD8^+$ en el paciente con IDVC en comparación con el control sano.

Los mitógenos son potentes estimuladores inespecíficos de la activación y proliferación de células T independientemente (170), por lo que las respuestas anormales de proliferación de las células T frente a mitógenos se consideran una prueba diagnóstica específica de la función aberrante de las células T (63,77,162,163,169). El método se realiza mediante el cultivo *in vitro* de células mononucleares de sangre periférica humana (PBMC) con lectinas de plantas mitogénicas (mitógenos) como fitohemaglutinina (PHA) y mitógeno de hierba carmín (PWM), comparando posteriormente, por ejemplo mediante citometría de flujo, los porcentajes de proliferación de los linfocitos T $CD4^+$ y $CD8^+$ del paciente comparado con los de un control sano.

Las pruebas de proliferación de linfocitos T frente a un antígeno específico se basan en la interacción *in vitro* de una CPA que procesaría un antígeno especifico (*feeder*) en la placa y lo presentaría al linfocito T, generando la activación y proliferación de las células T específicas de ese antígeno o bien mediante la cuantificación de linfocitos T memoria específicos para ese antígeno (171). Entre los principales antígenos utilizados se encuentran PPD, estreptoquinasa, CMV, antígeno de Candida y toxoide tetánico, que pueden tener resultados de proliferación subóptimos en un subgrupo de pacientes con IDVC (172).

Evaluación pulmonar: es esencial en la IDVC porque marca el pronóstico vital del paciente (26,98,101,173). A pesar de que no hay guías con consensos específicos sobre el despistaje, existen recomendaciones de expertos basadas en la experiencia clínica, con diversidad de publicaciones que lo avalan (174–176). Pruebas de función pulmonar como espirometría, test de difusión de CO y gases arteriales; estudio de imagen como radiografía de tórax, tomografía computarizada de alta resolución (TC) toraco-abdominal al diagnóstico, cada 5 años si no hay daño estructural ó cada 2 años si se identifica lesión, o bien resonancia magnética como opción alternativa si hay radio-sensibilidad (10,93,173). La realización de broncoscopia y lavado broncoalveolar se recomienda en caso de sospecha de enfermedad pulmonar granulomatosa (37,122).

Evaluación gastrointestinal: se debe realizar ecografía abdominal al diagnóstico, así como estudios de coprocultivo y parasitarios en todo paciente con IDVC, en especial en pacientes pediátricos con peso y talla por debajo del percentil adecuado para su edad (24,96,109). El test del aliento y endoscopia alta con biopsia en caso de sospecha de infección por *Helicobacter pylori* serían pruebas especiales a considerar (107,112,135).

En caso de clínica compatible con enteropatía "celíaca-*like*" es recomendable la realización de un estudio endoscópico con toma de biopsia. El estudio histopatológico se caracteriza por infiltración intraepitelial linfocítica, pérdida de vellosidades y criptas hiperplásicas (108). Los autoanticuerpos específicos asociados a celiaquía generalmente están ausentes y las anomalías histológicas no suelen responder a una dieta libre de gluten (177). Por otra parte, la hiperplasia nodular regenerativa del hígado está presente en más del 80% de los pacientes con IDVC, que se someten a una biopsia hepática (68).

Análisis genético: actualmente se considera que debería realizarse a todo paciente con diagnóstico establecido de IDVC de forma rutinaria (178). Existen varias herramientas para determinar el perfil de expresión genética de los pacientes, entre las que destacan principalmente: los *microarray*, la secuenciación *Sanger*, secuenciación masiva (NGS), la secuenciación del exoma completo (WES) o del genoma completo (WGS) (42,46,49,55,137,178,179). Independientemente del método utilizado, el estudio genético puede ser de ayuda para establecer un seguimiento adecuado que mejore el pronóstico e incluso de utilidad a la hora de realizar un consejo genético de estos pacientes.

Otros tests:

- Autoanticuerpos: a pesar de que las manifestaciones autoinmunes pueden estar presentes en hasta un 20% de los pacientes con IDVC (113,114), la medición de anticuerpos específicos asociados, de forma general, no son frecuentes. Se piensa que esto puede deberse como se comentó previamente, a un defecto intrínseco de producción de anticuerpos específicos o una respuesta aberrante frente a auto-antígenos por los T_{Reg} (2,20,67).

- Despistaje de linfoproliferación y de malignidad: el principal abordaje preventivo de malignidad en los pacientes con IDVC es el diagnóstico precoz de la misma, así como el tratamiento adecuado de las infecciones, en especial las asociadas a virus oncogénicos (107,119,127,130,135). En caso de sospecha de linfoproliferación, se recomienda la realización de pruebas de imagen dirigidas según cada caso, así como biopsia de ganglio linfático frente a la sospecha de linfoma. El IgRT no se ha visto asociado en una disminución del riesgo de malignidad en los pacientes con IDVC (131).

8.Manejo y Opciones terapéuticas de la Inmunodeficiencia Variable Común

8.1 Tratamiento Sustitutivo con Gammaglobulinas

El primer y más importante escalón de tratamiento a tener en cuenta en la IDVC es el IgRT (143,145,180,181). Si se inicia precozmente puede disminuir drásticamente la susceptibilidad

a infecciones bacterianas y víricas en especial en la esfera respiratoria, al disminuir la número y la gravedad de los cuadros infecciosos recurrentes, así como sus consecuencias en los pacientes susceptibles, mejorando claramente la morbilidad y pronóstico de la enfermedad (180). En pacientes que presentan niveles estables de Ig o ausencia de clínica infecciosa, el inicio del tratamiento puede posponerse, siempre que se lleve un seguimiento estrecho (143).

El IgRT puede llevarse a cabo por vía endovenosa (IVIG), vía subcutánea (SCIG) o bien SC facilitada (fSCIg), la decisión depende de las características clínicas específicas y preferencias del paciente, así como las recomendaciones de su médico especialista y la disponibilidad del centro hospitalario (181). La monitorización de los niveles séricos puede realizarse cada 3 a 6 meses, realizando ajustes correspondientes según las condiciones y necesidades del paciente, en especial aquellos que presenten comorbilidades asociadas a pérdidas de proteínas, mujeres embarazadas, variaciones significativas en el peso, etc. (143,145,182).

En general, el tratamiento es bien tolerado. Pueden encontrarse reacciones adversas en un 20%-50% de los pacientes, en su mayoría leves y más comunes durante la primera infusión, principalmente derivados de la dosis, la velocidad de infusión o estados protrombóticos basales (145,180,181). La presentación SC presenta menos reacciones adversas sistémicas que la IV, pudiéndose observar sin embargo reacciones locales como dolor y enrojecimiento de la zona de infusión (181). Las reacciones anafilácticas son excepcionales, en general presentan una incidencia entre 1,7 de 100.000 y 2,1 de 100.000 (183–185), que sin embargo, pueden comprometer la vida del paciente. Se considera que en pacientes con IDVC, la reacción puede estar mediada por formación de inmunocomplejos IgG o IgE anti-IgA, con consecuente activación del sistema del complemento (184). El tratamiento de las mismas deriva de los protocolos comunes, como protección de la vía área, oxígeno, antihistamínicos, glucocorticoides y/o epinefrina, según cada caso (186,187).

Existe evidencia de que el IgRT puede reducir la progresión de la enfermedad pulmonar y la frecuencia de infecciones graves, incluso ofrecer protección frente la contra procesos de autoinmunidad (10,144,150). No existe evidencia suficiente de que el IgRT pueda proteger contra el desarrollo de malignidad (129).

8.2 Tratamiento de Infecciones

En general, los pacientes con IDVC mejoran de forma clara la recurrencia y gravedad de infecciones al establecer el IgRT, como se ha comentado previamente (180). Sin embargo, un subgrupo de pacientes requieren ciclos largos de antibioticoterapia frente a infecciones bacterianas activas a pesar de adecuados niveles valle y personalizados para cada paciente (102,188). El tratamiento profiláctico con antimicrobianos no está recomendado de rutina, solo en caso específicos (148).

Como recomendación general, en todo paciente con diagnóstico de IDVC se debe descartar la infección por *Helicobacter pylori*, así como la presencia de anemia perniciosa, por su relación con cáncer gástrico (106,107,112). El tratamiento erradicador frente a *Helicobacter pylori*, es capaz de reducir y evitar la atrofia gástrica derivada de la infección crónica, así como el desarrollo de metaplasias, lo cual es el factor predisponente más importante para el posterior desarrollo de cáncer gástrico en estos pacientes (37,111,112).

8.3 Inmunizaciones

Las recomendaciones de vacunación en pacientes con IDVC van a depender de la severidad de la inmunodeficiencia (189). En general, el calendario de rutina debe incluir las vacunas inactivadas a la difteria, tétanos y vacuna *pertussis* acelular, vacuna frente a la hepatitis A y B, frente al *Haemophilus influenzae* tipo B, virus de papiloma humano, gripe estacional, meningococo, neumococo, polio (intramuscular) ántrax, encefalitis japonesa, rabia etc. (3,144,157,189,190). Las vacunas de virus vivos atenuados no están recomendadas en pacientes con deficiencia severa de anticuerpos (157,189).

8.4 Tratamiento de manifestaciones autoinmunes

El tratamiento de enfermedades autoinmunes en la IDVC es el mismo que en un paciente sin inmunodeficiencia asociada, acompañado siempre del tratamiento de reemplazo de inmunoglobulinas (113). En el caso de citopenias graves como trombocitopenia autoinmune o anemia hemolítica autoinmune, el tratamiento con glucocorticoides ocupa la primera línea de tratamiento (191). En caso de refractariedad, se puede considerar opciones de tratamiento inmunomodulador y/o immunosupresor como el Abatacept, Infliximab o Rituximab (86,191–193). Este último ha sido recomendado en pacientes con IDVC que expresen mutaciones en

CTLA-4 y LRBA (192). Los inhibidores mTOR puede ser una opción terapéutica en pacientes con defectos en la señalización de PI3K (194). En caso de falta de respuesta o curso tórpido, se podría valorar la indicación de esplenectomía o trasplante de progenitores hematopoyéticos (TPH), aunque estos últimos se reservan para caso extremadamente graves, siempre valorando el riesgo/beneficio, debido a la tasa de mortalidad de este procedimiento (118,167,195).

8.5 Tratamiento frente a Malignidad

Estos tratamientos se basan en protocolos similares a los aplicados en la población general (10,130,144,150,196). El aspecto más importante se basa en el seguimiento estrecho de los pacientes previo al desarrollo de malignidad, ya que presentan un riesgo 5 a 10 veces mayor de desarrollar neoplasias hematológicas principalmente (125,126,129). La prevención de cáncer asociada a infecciones como es el caso de ciertos linfomas y el cáncer gástrico, consiste en la prevención y tratamiento oportuno de las infecciones recurrentes, así como la realización de estudios virológicos, test del aliento, etc.

El tratamiento con el anticuerpo monoclonal anti-CD20 (Rituximab) está autorizado para el tratamiento del linfoma de linfocitos B. Estudios recientes han probado su eficacia y seguridad en distintas manifestaciones asociadas a IDVC, mostrando resultados prometedores (192,197). El TPH se realiza en pacientes con IDVC mayormente asociado a defectos en la inmunidad celular y en resistencia a terapias frente autoinmunidad (113,144,191). TPH es un tratamiento potencialmente curativo tanto de la inmunodeficiencia como de la malignidad hematológica.

8.6 Otras recomendaciones

- Las enfermedades pulmonares asociadas a pacientes con IDVC deben ser tratadas de forma individual, según cada caso.
- Las transfusiones sanguíneas o administración de componentes derivados de la sangre deben realizarse siempre bajo indicación de un especialista en inmunodeficiencias.
- Es necesaria la profilaxis antibiótica antes de la realización de un proceso mínimamente invasivo o procedimientos odontológicos.
- Los pacientes deben recibir consejo genético de cara a posible descendencia con riesgo de padecer inmunodeficiencia.

- Actualmente se están desarrollando nuevas dianas terapéuticas basadas en inmunomodulación y edición genética en busca de mejorar el pronóstico de pacientes con clínica severa.

9.Pronóstico de la Inmunodeficiencia Variable Común

El pronóstico de los pacientes con IDVC está condicionado principalmente por la existencia de complicaciones infecciosas, en especial las derivadas del daño pulmonar estructural crónico, así como el desarrollo de malignidades (linfoma y cáncer gástrico especialmente) (101,144,198,199). Una terapia adecuada y precoz puede prevenir o enlentecer su desarrollo (180,182,191,192). En general, la mortalidad acumulada en pacientes con IDVC, según edad y sexo, comparada con la población general es de aproximadamente el 20% (101).

En la actualidad las complicaciones derivadas de infecciones bacterianas han presentado una disminución drástica significativa debido al uso oportuno de tratamiento precoz con inmunoglobulinas (180,182). Entre complicaciones más frecuentes destacan las bronquiectasias hasta en un 20% de los pacientes (69,98,99). Los broncoespasmos, enfermedad pulmonar restrictiva u obstructiva y desarrollo de granulomas son complicaciones que también se han visto asociadas a estos pacientes (10,150,173).

Las complicaciones no infecciosas presentan una prevalencia del 60-70%, con un riesgo de muerte de hasta 11 veces mayor que la población general (5,119,200). En general, el riesgo estimado de desarrollo de cáncer se sitúa entre el 4% al 25% (107,125,126,129,131,134,135). Entre las malignidades más frecuentes el linfoma no Hodgkin (LNH) ocupa el primer lugar, en especial los de origen en linfocitos B, extranodal y bien diferenciados, con un riesgo acumulado de 2 a 8%, siendo más frecuentes en mujeres por encima de los 30 años (127,131,191). Entre los tumores sólidos en la IDVC, habría que destacar el cáncer gástrico asociado a infección por *H. pylori* (37,112,135). El diagnóstico tiende a realizarse en edades más tempranas en relación con la población general, con una histología asociada a adenocarcinoma moderado a pobremente diferenciado, con destacado número de linfocitos intratumorales (37,112).

OBJETIVOS

La **hipótesis** de esta memoria de tesis se basa en que la combinación de diversos biomarcadores (genéticos, bioquímicos e inmunológicos) diagnósticos y de respuesta terapéutica permitirá una mejor clasificación clínica y abordaje clínico de los pacientes con IDVC. Por consiguiente, el **objetivo principal** es primero, validar las clasificaciones clínicas existentes, para establecer y validar una escala basada en la combinación de biomarcadores con poder diagnóstico y pronóstico de la IDVC, que posibilite un seguimiento y tratamiento más personalizados de los pacientes. En paralelo a esta línea de investigación, se pretende profundizar en el conocimiento de los mecanismos biológicos y fisiopatológicos implicados en la inmunodeficiencia primaria sintomática más frecuente, lo que permitirá formular nuevas hipótesis direccionando nuevas investigaciones sobre esta enfermedad.

Los **objetivos específicos** son los siguientes:

I. Describir la epidemiología, presentación clínica, evolución y tratamiento de dos cohortes poblacionales de pacientes con IDVC de Portugal y España. Detallar los hallazgos del estudio genético de HLA de clase II, mediante NGS de un panel de genes relacionados con la IDVC y de secuenciación del exoma de los pacientes con clínica severa. Validar las escalas clínicas y terapéuticas para la IDVC en nuestra cohorte.

II. Definir el valor de las cadenas ligeras de inmunoglobulina en suero (sFLC) como biomarcador diagnóstico y pronóstico en IDVC.

III. Determinar el beneficio clínico de la profilaxis de infecciones recurrentes del tracto respiratorio mediante una vacuna polibacteriana de mucosas.

IV. Diseñar y validar una escala analítica pronóstica para pacientes con IDVC (Anexo).

DISCUSIÓN

Características de la Inmunodeficiencia Variable Común, Interrogantes y Áreas de Mejora.

La IDVC es una enfermedad altamente heterogénea clínica e inmunológicamente, denominada según el último consenso de expertos como un "síndrome", más que como una enfermedad definida. A pesar de que fue descrita por primera vez hace más de 60 años, a medida que se profundiza en su conocimiento, quedan numerosos interrogantes y áreas de mejora en los aspectos clínicos y científicos, convirtiéndola en el reto diagnóstico y terapéutico que es actualmente. Debido a que la carga genética solo se puede atribuir en un 15-20% a una mutación monogénica, un porcentaje similar a polimorfismos o a defectos derivados de modificaciones epigenéticas, en la mayoría de los pacientes no es posible encontrar la mutación asociada o bien los resultados de los estudios moleculares son no concluyentes, lo que subraya la relevancia de generar nuevas evidencias de herencia poligénica y metagenómicos, así como el camino que queda por recorrer en el conocimiento de la etiopatogenia de la IDVC.

Varios autores han criticado que los criterios de clasificación diagnósticos actuales podrían ser laxos o poco específicos (5,201–203), por lo que puede darse un sobrediagnóstico sindromático, a la espera de nuevas revisiones consensuadas considerando aspectos más "comunes" y menos "variables"(200).

A pesar de los avances en el manejo clínico de la IDVC, aún existe un retraso en el diagnóstico excesivo que condiciona la supervivencia de los pacientes. Nuestro estudio reveló un retraso en el diagnóstico de IDVC de 6 años, con un aumento de riesgo (3.7 veces mayor) de desarrollar linfoproliferación y/o malignidad, similar al descrito en otras cohortes (24,119). Este dato destaca la necesidad prioritaria de buscar estrategias de diagnóstico más precoz, que requieren la divulgación de los signos de alerta a otros especialistas para que los pacientes sean referidos a los inmunólogos clínicos de forma más precoz. Los biomarcadores descritos actualmente no permiten un diagnóstico diferencial claro con otras inmunodeficiencias, así como no son capaces predecir la totalidad de las manifestaciones clínicas asociadas a la enfermedad, por lo que son útiles de forma parcial para el diagnóstico y predecir la evolución, comportándose como datos aislados, no aportando una información integrada a una enfermedad tan compleja. Por está razón sería más útil establecer clasificaciones combinadas mediante escalas que permitan diagnosticar y predecir complicaciones, y por lo tanto actuar en tiempo real para modificar la historia natural de la enfermedad.

En cuanto a la acción terapéutica, a pesar de la mejora espectacular debida al tratamiento sustitutivo con gammaglobulinas (IgRT) en pacientes con IDVC en la incidencia y gravedad de las infecciones y por consiguiente, en las consecuencias derivadas de las misma, aún un subgrupo de pacientes con niveles de IgG valle adecuados presenta infecciones que condicionan su pronóstico y calidad de vida, siendo el repertorio de opciones terapéuticas eficaces escaso actualmente. Numerosos expertos destacan la necesidad de implantar nuevas estrategias beneficiosas y seguras en la búsqueda de reducir las infecciones y sus complicaciones, con mejoría en la calidad de vida.

En el marco de los interrogantes planteados y en una estrategia de explorar posibles nuevos biomarcadores que tengan utilidad clínica diagnóstica y pronóstica, que condicionen así medidas terapéuticas más personalizadas. Con este fin, el primer paso es conocer en profundidad la población de estudio con IDVC, de dos cohortes de pacientes con IDVC geográficamente diferentes. Una vez conocidas las características intrínsecas de nuestros pacientes, cuyos hallazgos más relevantes se señalan a continuación, se decidió aplicar las diferentes clasificaciones y escalas clínicas publicadas para la IDVC, lo que nos permitió por un lado dividir de forma más clara las diferentes expresiones clínicas de la enfermedad en nuestra cohorte, así como validar los diferentes sistemas de clasificación según los fenotipos clínicos de la IDVC propuestos y posteriormente revisados por Chapel et al. (5,114), la escala de severidad clínica propuesta por Ameratunga et al. (204) y el sistema de puntuación para guiar la decisión terapéutica de iniciar IgRT propuesta por Charlotte Cunningham-Rundles (143).

Validación de las Clasificaciones y Escalas Clínicas Propuestas por Grupos de Referencia en el Estudio de la IDVC.

Con respecto a los fenotipos clínicos de la IDVC, dos tercios de nuestros pacientes se incluían en una de las cuatro categorías, sin superposición. El tercio restante de nuestra cohorte mostró superposición de 2 fenotipos clínicos (principalmente citopenia e infiltración linfocítica policlonal), por lo que nuestros datos validan ampliamente este instrumento clinimétrico en poblaciones de diferente sustrato étnico y geográfico al original, al igual que otros grupos previos (5,182,200). La clasificación por fenotipos clínicos al momento del diagnóstico resulta de utilidad para el seguimiento específico de las complicaciones derivadas de forma individual. Sin embargo, el hecho de presentar en un momento específico un fenotipo particular no excluye

el desarrollo o solapamiento con otro fenotipo durante el curso clínico de la IDVC. La superposición de fenotipos clínicos puede sugerir mutaciones específicas responsables que podrían condicionar una evolución más severa de la enfermedad y llevar a cabo un estudio genético más dirigido.

En cuanto a la escala de severidad en la IDVC, a pesar de que no puede aplicarse como herramienta diagnóstica, tiene utilidad clínica permitiendo un seguimiento más estrecho de pacientes con afectación grave y ofrece información pronóstica relevante. En nuestra cohorte, las principales complicaciones graves se relacionaron con daño pulmonar (bronquiectasias principalmente), así como las asociadas a infecciones graves, como meningitis o septicemia, hallazgos similares a los descritos en otras cohortes de IDVC (97,98,123,204). Entre las complicaciones moderadas destacaron la neumonía complicada y alteraciones intestinales/ relacionadas con alteraciones nutricionales; y entre las leves, la otitis externa o media, así como la sinusitis aguda. Se concluye que este sistema de puntuación, a pesar de tener ciertas limitaciones (como la complejidad derivada del elevado número de ítems, a lo que se añade la superposición entre diferentes grados de complicaciones, el hecho de no identificar diferentes patrones de daño en los sistemas orgánicos y la variabilidad inter- o intra-observador), considera, no obstante, aspectos clínicos importantes que marcan la evolución de la IDVC y se recomienda su utilización en cohortes de forma global y como escala individual.

La instauración del IgRT según el sistema de puntuación propuesto por Charlotte Cunningham-Rundles (143) resulta asimismo una herramienta práctica fundamentalmente en los pacientes en los que la enfermedad se comporta de forma más leve y no es clara la necesidad de inicio de tratamiento. Esta escala se validó de forma retrospectiva en nuestra cohorte, donde todos los pacientes en IgRT cumplían los criterios de puntuación de IgRT, con una puntuación media de los criterios de laboratorio de 15 puntos y una puntuación clínica media de 20, resultando en una puntuación acumulativa de 35 puntos, lo cual indicaría la necesidad de tratamiento en nuestros pacientes. Estos resultados son similares a los resultados obtenidos en la cohorte descrita por Cunningham-Rundles (143) y valida aún más la utilidad de esta escala en la decisión de iniciar IgRT. Una desventaja de este sistema de puntuación radica en la necesidad de poseer cierta combinación de datos obtenidos de pruebas analíticas de laboratorio, medidas clínicas y hallazgos basados en el examen físico, donde existe un rango intermedio en el que

la decisión de instauración de tratamiento va a depender del médico examinador, lo que le da cierta subjetividad.

Toda vez realizada una descripción exhaustiva de las cohortes y validada según las escalas estandarizadas para IDVC, se planteó la hipótesis de trabajo, que pretende aportar nuevos biomarcadores diagnósticos, marcadores pronósticos útiles en una aproximación más integral a la enfermedad, así como proponer nuevas opciones de tratamiento en una subpoblación susceptible de infecciones a pesar de la terapia adecuada, así como contribuir al avance en el conocimiento de la fisiopatología de la IDVC.

Propuesta de Biomarcadores Diagnósticos en la Inmunodeficiencia Variable Común.

- Se propone a las sFLC como marcador diagnóstico diferencial de la Inmunodeficiencia Variable Común frente otras IDP e IDS. Así como un nuevo criterio diagnóstico de la IDVC.

En nuestra cohorte de pacientes con IDVC, los valores de sFLC κ y/o λ fueron significativamente más bajos en comparación con otras IDP e IDS ($p < 0,001$) (en torno a 10 veces menores), hallazgo apoyado por resultados en cohortes previas (136,205,206). Sin embargo, para definir mejor el nivel de corte más óptimo para sFLC en nuestra población, se empleó la adición de los valores de κ y λ (SUMA κ+ λ) y se aplicó la metodología de curvas ROC para evaluar la capacidad de discriminar IDVC frente a otras inmunodeficiencias, estableciendo un punto de corte idóneo de <16,7 mg/L, con un AUC de 0.894, una sensibilidad de 92%, una especificidad de 75% y un VPN del 98% para el diagnóstico de IDVC frente a otras IDP así como IDS. El riesgo relativo (RR) de presentar IDVC para los pacientes que presentaban una SUMA κ+λ por debajo de 16,7 mg/L fue 20,35 veces mayor (95%, IC: 5,630–75,93) que los pacientes con valores menores a 16,7. El comportamiento característico de sFLC en los pacientes con IDVC, más aplicando este punto de corte, nos permite considerar y por lo tanto proponer la utilización de las sFLC como marcador diagnóstico diferencial de la IDVC con otras IDP e IDS.

Propuesta de Marcadores Pronósticos en la Inmunodeficiencia Variable Común.

Junto de la validación de las escalas pronósticas descritas de fenotipos y severidad existentes para la IDVC (5,204) y de la estratificación de los pacientes con IDVC según el biomarcador de la subpoblación de células B memoria con cambio de isotipo, se proponen nuevos biomarcadores asociados a complicaciones específicas, especialmente el desarrollo de malignidad, con el diagnóstico precoz y preciso, estableciendo un seguimiento más adecuado. El presente trabajo de tesis propone los siguientes biomarcadores predictivos o asociados a complicaciones clínicas:

- Se propone el estudio de HLA de clases II en los pacientes con Inmunodeficiencia Variable Común, como marcador pronóstico en el desarrollo de Autoinmunidad y Enteropatía Inexplicada.

El estudio de la expresión de HLA de clase II de nuestra cohorte de pacientes con IDVC mostró una frecuencia de los haplotipos DR4, DR7, DR11y DQ5 asociados significativamente con la susceptibilidad de presentar ciertas enfermedades como la autoinmunidad. Este hallazgo se ha descrito como un factor predisponente de autoinmunidad independiente de la presencia de la inmunodeficiencia (1,1,2). Gracias al trabajo pionero de Noel R. Rose, fallecido este mes de agosto de 2020, sobre la base genética de las enfermedades autoinmunes, se conoce la relación etiopatogénica del CMH con el riesgo a padecer ciertas enfermedades autoinmunes (207,208), en el contexto de la presentación antigénica de ciertos péptidos por las APC a los linfocitos T del paciente, lo que determina la variabilidad de la respuesta inmunológica de cada individuo (1,207,209–211). Aunque la realización de rutina del estudio de expresión de HLA no está justificado en la población general, los pacientes con IDVC presentan hasta un 20% de susceptibilidad de presentar enfermedades autoinmunes asociadas, en especial trombocitopenia autoinmune y anemia hemolítica autoinmune, según los hallazgos descritos en las diferentes cohortes de IDVC publicadas, como el fenotipo clínico no infeccioso más prevalente en esta enfermedad (5,182,200), deja abierta la posible utilidad de incluir este estudio como factor predictor de autoinmunidad en IDVC. De la misma forma, la expresión de marcadores HLA de susceptibilidad a la enfermedad celíaca (DQ2.5 y DQ2.2) asociados al desarrollo de manifestaciones GI no infecciosas con autoanticuerpos negativos (enfermedad celiaca-like), no ha sido descrita previamente en los pacientes con IDVC. En nuestra cohorte, el 80% de los

pacientes IDVC con clínica GI no infecciosa expresaban estos marcadores de predisposición, asociación altamente significativa frente a los pacientes sin clínica GI o frente a la población general. Si bien las moléculas HLA-clase II DQ2.5 y DQ2.2 son condición necesaria pero no suficiente para el desarrollo de la enfermedad celiaca (212,213), el estudio de estas moléculas al diagnóstico podría ser una herramienta útil para la aproximación clínica en este grupo de pacientes. De cara a este hallazgo, consideramos igualmente que en los pacientes que presentan la expresión de HLA de susceptibilidad de celiaquía y clínica GI no infecciosa asociada, sería interesante realizar un estudio más especifico mediante el análisis de los linfocitos intraepiteliales (LIE) en biopsia duodenal, a pesar de la ausencia de autoanticuerpos frente a transglutaminasa, endomisio y PPG. La especificidad de la biopsia no está del todo clara en los pacientes con IDVC con manifestaciones GI (108,214). En cualquier caso, el tratamiento deberá consistir en instaurar una dieta libre de gluten y un seguimiento digestivo estricto. En caso de que no presentar mejoría tras la suspensión del gluten de la dieta, debe considerarse la presencia de una enteropatía autoinmune y plantear tratamiento con terapia inmunosupresora. Igualmente, el estudio del microbioma en estos pacientes puede arrojar luz en los mecanismos fisiopatológicos y se está considerando para nuevas estrategias de abordaje terapéutico (215). Este aspecto requiere validar nuestros resultados en una población más amplia.

- El Estudio de Activación de los Linfocitos T $CD8^+$ se correlaciona con otros biomarcadores de riesgo de Autoinmunidad en pacientes con Inmunodeficiencia Variable Común.

En nuestro estudio se detectó que el aumento de HLA-DR^+ en la superficie de los linfocitos T $CD8^+$ se correlacionaba inversamente con la expresión de $CD45RA^+$; y la población de linfocitos T de memoria se correlacionaba inversamente con los linfocitos B de memoria con cambio de isotipo ($p<0.05$ ambos). La expresión de HLA-DR^+ en los linfocitos T se considera como un marcador de su estado de activación (216), lo que puede estar reflejando un exceso de co-estimulación entre los linfocitos T-B en el contexto de enfermedad autoinmune. Esta expresión de linfocitos T $CD8^+$ activados podría deberse a la presencia de una infección subclínica crónica, asociada a una alteración del microbioma intestinal en pacientes con IDVC, como se ha sido hipotetizado por algunos autores (29,32,75). También podría sugerir una alteración en la funcionalidad de la molécula de HLA-DR^+ en los linfocitos T $CD8^+$ vinculada a una expresión específica aberrante ubicada a nivel genético (Cromosoma 6) (1,165,209). Debido a que los linfocitos T $CD8^+$ tienen un papel efector final en muchas enfermedades

autoinmunes, este dato resulta especialmente interesante y sería un aspecto a explorar la relación biológica subyacente a esta asociación con el fenotipo memoria B. De la misma forma, la función supresora de T_{Reg} estaba significativamente disminuida en nuestra cohorte de IDVC, lo que podría estar asociado igualmente a la patogénesis de la enfermedad autoinmune en estos pacientes, si bien en nuestra cohorte no se encontró una correlación directa ni significativa entre ambas, probablemente debida al tamaño muestral.

El estudio de fenotipo de linfocitos T en pacientes con IDVC nos permitiría determinar el estado de activación de las distintas subpoblaciones de linfocitos T, responsables en gran medida del desarrollo de las manifestaciones autoinmunes, lo que nos permitirá instaurar o seleccionar mejores terapias inmunomoduladoras dirigidas en estos pacientes.

- Clasificación de los patrones de sFLC en la Inmunodeficiencia Variable Común como marcadores pronósticos de manifestaciones por fenotipos clínicos.

En nuestros pacientes con IDVC se observó una correlación entre el patrón $\kappa^{-}\lambda^{+}$ y la presencia de una mayor incidencia de infecciones respiratorias superiores e inferiores. Asimismo, se encontró que la enteropatía, esplenomegalia y bronquiectasias fueron más prevalentes en el patrón $\kappa^{-}\lambda^{-}$ en nuestra cohorte, si bien la ausencia de significación estadística con esplenomegalia y bronquiectasias podría deberse al pequeño tamaño de nuestra serie. Scarpa et al. también analizaron la asociación de patrones de sFLCs con los fenotipos clínicos de la IDVC, y contraintuitivamente, los fenotipos infecciosos e inflamatorios se observaron con mayor frecuencia en pacientes con IDVC con niveles bajos o ausentes de sFLC ($\kappa^{-}\lambda^{-}$) (6, 7, 29). En el estudio de Compagno et al. el patrón de sFLCs más representado en la IDVC fue $\kappa^{-}\lambda^{+}$ con mayor riesgo de mortalidad derivado de citopenias autoinmunes, linfoproliferación y enteropatía, seguido del patrón $\kappa^{-}\lambda^{-}$ que presentó tendencia a desarrollar esplenomegalia y malignidad (6). En conjunto, estos hallazgos apoyan que los valores disminuidos de sFLC observados en la IDVC están asociados con las diferentes manifestaciones clínicas observadas en esta patología y pueden utilizarse como una herramienta pronóstica accesoria. Sin embargo, la importancia clínica de estos patrones aún está en estudio y necesita una mayor validación en series más amplias de pacientes.

- Determinación y seguimiento del cociente de sFLC κ/λ como marcador pronóstico de desarrollo de síndrome linfoproliferativo B en pacientes con Inmunodeficiencia Variable Común.

Debido a la predisposición a desarrollar malignidad, así como el patrón tan característico de las sFLC de los pacientes con IDVC, se sugiere que, ante un paciente con malignidad hematológica, la presencia de valores bajos o indetectables de κ y/o λ podrían estar apuntando a una IDVC subyacente. Este dato es especialmente relevante, puesto que en un porcentaje de pacientes con IDVC, se sabe que un síndrome linfoproliferativo B puede ser la manifestación de inicio de la enfermedad, y que una vez instaurado el tratamiento del cáncer hematológico, las posibilidades de diagnosticar al IDVC son muy bajas. El diagnóstico de IDVC en el contexto de cáncer va a condicionar el tratamiento, que debería ser ajustado a la inmunodeficiencia (dosis más bajas, por ejemplo), y a la incidencia de mayor riesgo de infecciones, de recurrencias del cáncer e incluso de segundas neoplasias, por lo que el papel y las investigaciones en esta dirección son de gran importancia clínica. Sin embargo, se desconoce el porcentaje de pacientes con malignidad hematológica que son IDP, los datos epidemiológicos globales apuntan que hasta un 20% de los cánceres se asociarían a infecciones persistentes por gérmenes oncogénicos.

Por otra parte, en los pacientes ya diagnosticados de IDVC, una relación κ/λ alterada o un aumento repentino en los valores de sFLC podrían ser un indicador de la necesidad de realización de estudios adicionales, que permitan estrategias terapéuticas precozmente. Una limitación de este estudio es el hecho de poseer una cohorte con un tamaño muestral reducido, donde la variabilidad de las sFLC fue medida solo en dos tiempos. Sin embargo, debido a los resultados obtenidos en nuestro estudio consideramos que deben diseñarse estudios específicos de determinación seriada y seguimiento a largo plazo de los niveles de sFLC en pacientes con IDVC que presenten factores de riesgo para el desarrollo de malignidad, así como el estudio de estos marcadores en pacientes con malignidad hematológica establecida para demostrar esta relación, que es válida para la población general. Todo lo anterior apoya la utilidad de las sFLC como herramienta accesoria diagnóstica y pronóstica en los pacientes IDVC.

- Propuesta de una Escala Pronóstica Combinada para la Inmunodeficiencia Variable Común: Variable Immunodeficiency Score Upfront Analytical Link (VISUAL).

Se propone una escala pronóstica combinada para la IDVC. Como se ha mencionado, la única escala de laboratorio es la propuesta por Cunningham-Rundles para la decisión de IgRT, así como el biomarcador aislado del fenotipo de memoria B como predictor pronóstico, que engloba de forma parcial las posibles manifestaciones clínicas asociadas, por lo que no representan una imagen completa de la enfermedad y la evolución individual de cada paciente. La aplicación de escalas definidas para la enfermedad representa una evaluación más integral de la expresión clínica en comparación con los datos aislados, como se ha demostrado por la mayor capacidad de discriminación por curvas ROC, que requerirá su validación en otras cohortes de pacientes con IDVC.

La escala VISUAL (Variable Immunodeficiency Score Upfront Analytical Link) utiliza 4 biomarcadores inmunológicos combinados (linfocitos B de cambio de isotipo, IgA, respuestas a anticuerpos específicas y linfocitos T $CD4^+$), realizados de forma rutinaria en el momento del diagnóstico que permiten predecir de forma temprana la gravedad de manifestaciones clínicas en los pacientes con IDVC, independientemente del curso de la enfermedad. Esto hace que su utilización sea sencilla y pudiera generalizarse, en caso de confirmarse su utilidad en series independientes. Es una herramienta de cribado no invasiva y fácil de realizar que permitiría identificar a los pacientes con IDVC en riesgo de presentar complicaciones graves y que se beneficiaría de un seguimiento clínico y terapias más personalizadas, en el contexto de una medicina de precisión. Nuestros resultados apoyan la hipótesis de que las manifestaciones clínicas y, por tanto, el mayor riesgo de complicaciones en los pacientes con IDVC, se asocian con una alteración más profunda de los biomarcadores analíticos en el momento del diagnóstico que los que hay descritos hasta el momento.

A pesar de que es complicado establecer una distribución lineal en todos los pacientes en un escenario de la vida real, y que el rendimiento de cualquier instrumento debe evaluarse por separado en individuos con inactividad o actividad leve de la enfermedad de los pacientes con actividad de la enfermedad moderada a grave, la escala VISUAL parece ser una herramienta útil para clasificar a los pacientes con IDVC al momento del diagnóstico con el fin de anticipar y ajustar el seguimiento y el tratamiento de los pacientes. Esto se representa en la corrección lineal que presenta esta escala ajustándose de forma significativa con la escala de severidad

propuesta por Ameratunga et al. (204), donde observamos que nuestra cohorte presentó mayor escala VISUAL a medida que presentaba mayor puntaje en la escala de severidad. La contribución más interesante de VISUAL es la correlación entre la puntuación de los biomarcadores analíticos al momento del diagnóstico que puede ayudar a determinar tempranamente el posible pronóstico de cada paciente, así como la gravedad de las manifestaciones clínicas.

La escala VISUAL se correlacionó con la escala clínica pronóstica establecida actualmente. VISUAL ≥10 puntos tiene una sensibilidad del 85% y un VPN del 77% de identificar al diagnóstico los casos graves, significativamente superior al porcentaje de linfocitos B de memoria con cambio de isotipo de forma aislada (p=0.01). VISUAL por tanto integra información inmunológica para la categorización de la IDVC más allá de un biomarcador aislado (12,66) y que se correlaciona significativamente con la escala clínica pronóstica propuesta por Ameratunga y estandarizada. Es importante considerar que los linfocitos B memoria con cambio de isotipo no son capaces de predecir el desarrollo de complicaciones infecciosas graves, como sí lo hacen el defecto en la producción de anticuerpos específicos ó los niveles séricos de IgA. Una de las debilidades de este estudio es el tamaño relativamente pequeño de la cohorte. Sin embargo, debido a los resultados obtenidos, sería de interés validar VISUAL en una cohorte más amplia de pacientes.

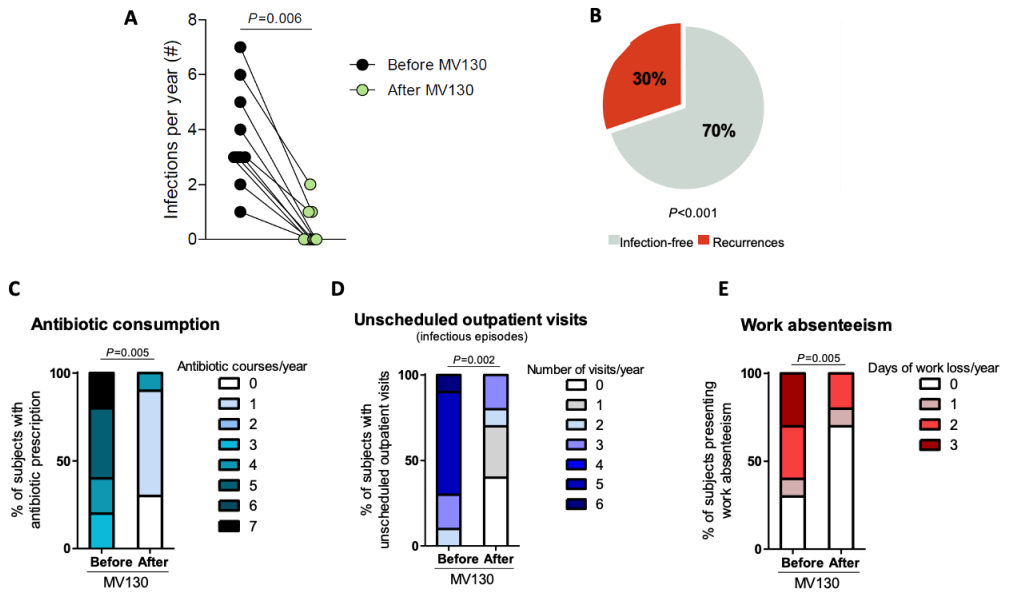
Aportaciones en el Tratamiento de la IDVC. Vacunas Basadas en Inmunidad Entrenada como Estrategia Profiláctica en la Inmunodeficiencia Variable Común.

A pesar de las aportaciones de Cunningham-Rundles en la decisión de inicio terapéutico con gammaglobulinas (143), todos los grupos expertos en IDVC reconocen la existencia de un subgrupo de pacientes con tratamiento adecuado y niveles de IgG valle ajustados que sin embargo, continúan presentando infecciones recurrentes o subclínicas, siendo ésta una población más vulnerable al desarrollo de complicaciones que afectan de forma directa su calidad de vida. Las opciones terapéuticas para estos pacientes son escasas, por lo que, uno de los objetivos de este trabajo fue proponer una opción terapéutica alternativa válida para esta población.

Una reciente publicación de Quinti et al. (148), propone un protocolo de antibióticoterapia profiláctica con dosis bajas de azitromicina para la reducción de episodios de exacerbación de

infecciones respiratorias por paciente-año en estos pacientes, con la consiguiente reducción de ciclos adicionales de antibióticos y menor riesgo de hospitalización. Se trata del primer ensayo clínico de profilaxis antibiótica en pacientes con IDVC. No obstante, la antibiótico-profilaxis tiene limitaciones, como expresan los mismos autores, entre las que destacan el riesgo de colonización bacteriana, desarrollo de neumonía complicada, infecciones invasivas o infecciones por microorganismos atípicos no fue completamente estudiado. Igualmente, a pesar de que no está claro si los pacientes con IDP son más susceptibles de desarrollar resistencia a los antimicrobianos, este aspecto representa un importante problema de salud en todo el mundo (217–219). Además, los antibióticos tienen limitaciones frente a diversas infecciones, principalmente víricas, son perjudiciales para el microbiota (ya de por sí alterada en los pacientes con IDVC) y no están libres de efectos adversos a largo plazo. En este contexto, estrategias complementarias o alternativas para prevenir infecciones en esta población de alta susceptibilidad es una prioridad.

Nuestra unidad clínica es una unidad de referencia de la IDVC en la Comunidad de Madrid, en la que se siguen pacientes según quías de práctica clínica en las IDP, con seguimiento estrecho y personalizado de los pacientes. Se llevó a cabo un estudio observacional de prueba de concepto de la utilización de una vacuna basada en inmunidad entrenada **(**TIbV), formada por una fórmula polibacteriana MV130, para pacientes con infecciones recurrentes del tracto respiratorio (RRTI). MV130 disminuyó significativamente la tasa de infección (p=0,006) durante los 12 meses posteriores al inicio del tratamiento con respecto al año previo, así como una disminución significativa en la administración de antibióticos y de visitas ambulatorias no programadas debidas a infecciones (**Figura 10).** Se detectaron aumentos significativos en los anticuerpos IgA anti-neumococo y frente al propio preparado MV130 en suero (p=0,039 ambos) a los 12 meses de tratamiento con MV130. Esto implica un beneficio clínico en infecciones probablemente hetrólogas, que era lo que se pretendía, acompañado de una respuesta específica a los componentes de la vacuna, en el contexto de una enfermedad que se define como déficit de respuesta humoral, por lo que ésta última respuesta es especialmente relevante. A pesar de que la principal debilidad de este estudio es el diseño observacional retrospectivo, que puede resultar en la pérdida de variables no registradas en la práctica clínica habitual, los resultados obtenidos son prometedores y podrían ayudar en el diseño de un ensayo clínico.



(**Figura 10).** La profilaxis con MV130 reduce significativamente la incidencia de infecciones respiratorias (A – B), así como la tasa de consumo de recursos sanitarios y el absentismo laboral. (C – E). (A) Número de episodios infecciosos del tracto respiratorio puntuados 1 año antes de la inmunización (negro) y en los 12 meses posteriores al inicio de la inmunoterapia con MV130 (verde). (B) Porcentaje de sujetos que permanecieron libres de infección (gris) o sufrieron recurrencias (rojo) en los 12 meses posteriores a la administración de MV130. Las barras muestran el número relativo de (C) Consumo de antibióticos, (D) visitas a urgencias y (E) y absentismo laboral durante el año anterior y posterior al inicio del tratamiento con MV130 en el total de sujetos registrados. Modificado de Guevara-Hoyer et al. 2020 (220)

La inmunidad entrenada se basa en procesos biológicos derivados de la memoria innata asociada con señales intracelulares que impulsan cambios metabólicos profundos y modificaciones epigenéticas que dan como resultado la reprogramación de células de inmunidad innata (43,44). En los pacientes con IDVC, se ha descrito defectos a nivel de vías de señalización como TLR y NLR (73,74,221–223). MV130 puede inducir la inmunidad innata mediante la activación de estas vías de señalización en células dendríticas humanas, secretando citoquinas como TNF-α, IL-6 e IL-1β relacionadas con la inmunidad entrenada. Estas células dendríticas poseen la capacidad de promover respuestas Th1 y Th17 a través de la vía de señalización RIPK2 y MyD88 bajo el control de IL-10, con una gran cantidad de receptores de reconocimiento de patrones que facilitan la acción sinérgica de los sistemas inmunológico innato y adaptativo (224).

Apoyando nuestros resultados, MV130 ha demostrado activar la inmunidad entrenada *in vitro* y *ex vivo* (225,226), con el potencial de mejorar la respuesta inmunológica al proteger frente a infecciones secundarias, y revertir los estados de inmunotolerancia y las afecciones

inflamatorias crónicas (227–229), como las exacerbaciones respiratorias en el contexto de la enfermedad pulmonar obstructiva crónica (225,230,231). En pacientes con RRTI sin una IDP definida, MV130 ha demostrado no solo beneficios clínicos, sino una respuesta específica anti-infecciosa (neumocócica) y no específica (antiviral) (231,232). La vía sublingual para la administración de preparaciones bacterianas inactivadas se ha propuesto como una inmunoterapia segura, efectiva y duradera para estimular la inmunidad humoral y celular (220,231).

De la misma forma, Quinti et al. que se han esforzado en determinar cuales son los aspectos que más afectan la calidad de vida de los pacientes con IDVC, diseñando un cuestionario fácil de aplicar durante el seguimiento de los pacientes (233), han expuesto que las RRTI tienen un efecto crítico en la calidad de vida de los pacientes con IDVC (233). Según nuestros resultados, la intervención profiláctica de vacunas basadas en inmunidad entrenada muestra una mejoría de la percepción global de la calidad de vida en más de la mitad de los pacientes con IDVC, mejorando una media de hasta 17% hasta un 50% con respecto a la puntuación del score previo al tratamiento. La "dificultad en las actividades habituales" fue el componente con la mayor disminución en la puntuación posterior al tratamiento, así como, "miedo a perder la medicación" fue el único elemento que obtuvo una puntuación más alta después de la intervención profiláctica. Estos hallazgos resaltan aún más el impacto positivo de la profilaxis con un TIbV como MV130 en la calidad de vida de los pacientes con IDVC.

En cuanto al estudio fármaco-económico, la disminución de la frecuencia y la gravedad de las infecciones entre los pacientes con IDVC después de instaurar IgRT se traduce en un ahorro de gasto de la atención médica (180,181,234). En nuestro estudio, excluyendo el gasto del tratamiento con IgRT, la terapia de inmunización sublingual con MV130 se estimó en una reducción anual de aproximadamente 9.000–14.000 €/paciente, lo que apoya que la inmunoestimulación bacteriana podría ser una estrategia efectiva para reducir los costos y favorecer el control de la infección subclínica, así como las exacerbaciones infecciosas del tracto respiratorio en pacientes con IDVC. Nuestros datos preliminares en una pequeña cohorte de pacientes con IDVC con RRTI recurrentes muestran que la inmunización con TIbV es una estrategia beneficiosa y segura para reducir las infecciones. La propuesta de este enfoque terapéutico podría representar un elemento clave para el control de la infección subclínica en pacientes con IDVC con mejoría en la calidad de vida y consecuencias económicas positivas.

Aportaciones al Conocimiento de la Fisiopatología de la IDVC. Cadenas Ligeras de Inmunoglobulinas en Suero: ¿La Característica Común de la Inmunodeficiencia Variable Común?

La causa de la alteración constatada de las sFLC en los pacientes con IDVC no ha sido claramente dilucidada. Se proponen varias hipótesis que pudiesen explicar este fenómeno. Por un lado, los niveles disminuidos de sFLC podrían estar condicionados, ya sea por una mayor eliminación a nivel renal u otra vía de pérdida de proteínas o por una baja secreción por parte de las células plasmáticas. Ambas causas, aunque posibles, las consideramos poco probables como los responsables de este proceso, debido al hecho de que en nuestra cohorte ningún paciente presentó pérdida renal u otra pérdida de proteínas asociada, y que aunque cuantificar las células plasmáticas en la médula ósea, no sea factible, el reflejo de su función, medido de forma indirecta por el número total de linfocitos B periféricos, los linfocitos B con cambio de isotipo, así como la IgG sérica total no presentaron una asociación clara con los niveles de sFLC en nuestros pacientes con IDVC.

Considerando las alteraciones de desarrollo celular y el fenotipo clínico de los pacientes con IDVC, consideramos que la causa más probable que explicaría este fenómeno podría deberse a un reordenamiento alterado, ensamblaje o secreción de cadenas ligeras durante la ontogenia de linfocitos B o bien a un defecto intrínseco en la secreción de cadenas ligeras por parte de las células plasmáticas. Los genes Ig se reorganizan, primero en el desarrollo temprano de los linfocitos B, a través de la recombinación V (D) J en el hígado, posteriormente en la médula ósea y aún más tras el encuentro con el antígeno a través del proceso de hipermutación somática (SHM) en los centros germinales de los ganglios linfáticos. Estas dos últimas hipótesis implican que los bajos niveles de sFLC en pacientes con IDVC pueden reflejar una alteración intrínseca que afecta la producción, el ensamblaje o la secreción normal de las cadenas ligeras en las moléculas de Ig, lo que apunta a defectos específicos en la diferenciación de las células plasmáticas (235), que se remonta a un evento temprano crítico durante la diferenciación de linfocitos B (**Figura 9).**

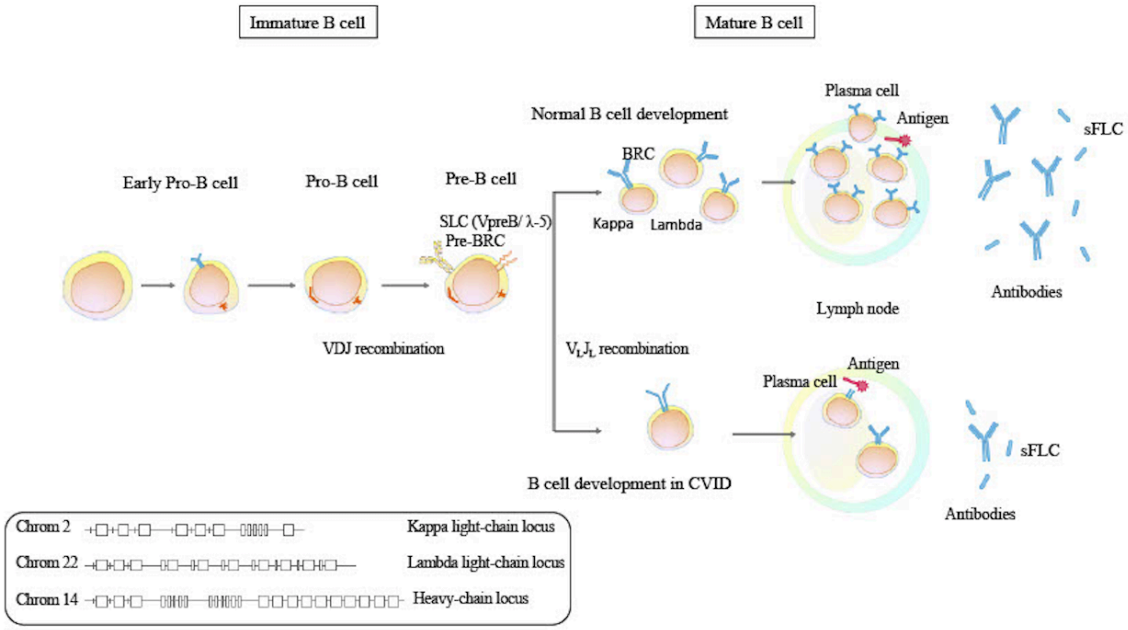


Figura 9. Puntos críticos en el desarrollo de la síntesis, el ensamblaje y la secreción de sFLC. Esquema de maduración y diferenciación de las células B, donde una alteración en el reordenamiento de los receptores de las pre-células B, podría condicionar la producción inadecuada de cadenas ligeras de inmunoglobulinas y, en consecuencia, la expresión defectuosa de inmunoglobulinas séricas. Adaptado de Winkler y Mårtensson (236).

Se está trabajando actualmente en un proyecto para verificar las hipótesis que enraízan este fenómeno, descartando defectos genéticos que afecten a la ontología temprana de células B, o alteraciones intrínsecas en la diferenciación de células plasmáticas terminales, mediante la diferenciación *in vitro* de células plasmáticas de pacientes con IDVC tras la estimulación, con posterior determinación de la producción y secreción de sFLC, así como, estudios a nivel genético en puntos críticos específicos que pudieran explicar el comportamiento tan característico de la sFLC en la IDVC.

El conjunto de datos proporciona nueva evidencia de las alteraciones biológicas subyacentes a la IDVC y deja completamente abierta nuevas avenidas de investigación, así como la cuestión de si sería necesario revisar la clasificación de IDVC de acuerdo con estos resultados.

CONCLUSIONES

Los resultados de este trabajo nos permiten concluir:

1. A medida que se profundiza en el conocimiento de la IDVC, se identifican numerosos interrogantes y áreas de mejora en diversos aspectos científicos y clínicos, que la convierten en el reto diagnóstico y terapéutico que es actualmente.

2. El debut de la enfermedad, los gérmenes implicados en las manifestaciones infecciosas y el perfil de activación inmunológica asociado a las manifestaciones no infecciosas fueron las principales diferencias encontradas entre las cohortes estudiadas, sin embargo, estás diferencias apuntan a ser un reflejo intrínseco de la IDVC, no necesariamente asociados a un grupo poblacional específico.

3. Nuestro estudio reveló la relación de un retraso en el diagnóstico de IDVC de 6 años, con un aumento de riesgo de 3.7 veces mayor de desarrollar linfoproliferación y/o malignidad, similar al descrito en otras cohortes.

4. Las sFLC se comportan como un biomarcador distintivo en el diagnóstico diferencial de la IDVC frente a otras PID y SID, reafirmando un potencial defecto ontogénico de los linfocitos B en esta patología. Se propone la medición de sFLC como criterio diagnóstico de pacientes con IDVC.

5. Se propone la variable de "suma κ+λ" como la más optima de las sFLC para el diagnóstico de IDVC. Mediante curva ROC, el punto de corte suma κ+λ <16,7 mg /L permite el diagnóstico de IDVC frente a otras PID y SID con una sensibilidad del 92%, especificidad del 75% y un Valor Predictivo Negativo de 98%.

6. El estudio de expresión de HLA de clase II de nuestra cohorte de pacientes con IDVC, mostró una frecuencia de haplotipos relacionados significativamente con la susceptibilidad de presentar enfermedad autoinmune independiente de la presencia de la inmunodeficiencia, marcador que podría ser utilidad como factor predictor de autoinmunidad en IDVC.

7. El HLA de clase II de susceptibilidad a enfermedad celíaca (DQ2.5 y DQ2.2) se expresó significativamente en el 80% de los pacientes con IDVC y manifestaciones GI no infecciosas, asociación no descrita en la literatura. El análisis de estos haplotipos al diagnóstico puede predecir complicaciones clínicas GI en la IDVC.

8. La sobreexpresión de HLA-DR^+ en la superficie de linfocitos T $CD8^+$ se correlacionó significativamente con la presencia de enfermedad autoinmune, así como la disminución de la función supresora de T_{Reg} en nuestra cohorte de IDVC. Estos hallazgos requieren validación como posibles biomarcadores predictores de enfermedad autoinmune en IDVC.

9. El cociente κ/λ se propone como un biomarcador pronóstico de la IDVC asociado a fenotipos clínicos específicos, que puede alertar precozmente la presencia de una malignidad linfoide, permitiendo un diagnóstico y una terapia adecuados en el paciente individual.

10. Se propone la escala VISUAL (Variable Immunodeficiency Score Upfront Analytical Link**)** para el diagnóstico de IDVC, dirigido una orientación clínica y seguimiento estrecho de los casos con VISUAL >10 puntos, enfocados a un tratamiento más individualizado y el desarrollo de estrategias de seguimiento más personalizadas. Esta herramienta de puntuaciones superior al biomarcador aislado de linfocitos B de memoria con cambio de isotipo (EUROClass) en sensibilidad y precisión diagnóstica de casos graves.

11. En un estudio observacional de prueba de concepto, la profilaxis con TIbV MV130 perlingual basada en bacterias inactivadas disminuyó significativamente la frecuencia de infecciones, las visitas inesperadas por infección y la utilización de antibioticoterapia con un perfil de seguridad adecuado en los pacientes con IDVC y RRTI a pesar de niveles valle adecuados de IgG.

12. La vacuna TIbV MV130 mejoró de forma concomitante la percepción de la calidad de vida del paciente según el cuestionario “The CVID_QoL Questionnaire” y el coste sanitario en los pacientes con IDVC e RRTI.

Este trabajo permite la integración de biomarcadores clínicos, biológicos, inmunológicos, de estilo de vida y genéticos, que conforman la base de una asistencia sanitaria personalizada, en pro de profundizar en el conocimiento de esta enfermedad y predecir el espectro de complicaciones y progresión de la IDVC, postulando el diseño de nuevos algoritmos que permitan brindar un tratamiento más personalizado y estrategias de seguimiento dirigidas.

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ANEXOS

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Variable immunodeficiency study: Evaluation of two European cohorts within a variety of clinical phenotypes

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ABSTRACT

Introduction: Given the wide heterogeneity of common variable immunodeficiency (CVID), several groups have proposed clinical and immunological classifications to better define follow-up and prognostic algorithms. The present study aims to validate recent clinical and laboratory algorithms, based on different combinations of CVID biomarkers, to provide more personalized treatment and follow-up strategies.

Methods: We analysed clinical and immunological features of 80 patients with suspected or diagnosed CVID, in two reference centres of Portugal and Spain. Clinical manifestations were categorized into clinical phenotyping proposed by Chapel et al. [1] that included cytopenia; polyclonal lymphocytic infiltration; unexplained enteropathy; and no disease-related complications.

Results: 76% of patients in our cohort entered one of the four categories of clinical phenotyping, without overlap (cytopenia; polyclonal lymphocytic infiltration; unexplained enteropathy; and no disease-related complications). The most prominent phenotype was “cytopenia” (40%) followed by “polyclonal lymphocytic infiltration” (19%). The remaining 24% patients of our cohort had overlap of 2 clinical phenotypes (cytopenia and unexplained enteropathy mainly). A delay of CVID diagnosis in more than 6 years presented 3.7-fold higher risk of developing lymphoproliferation and/or malignancy ($p < 0.05$), and was associated with increased $CD8^+CD45RO^+$ T-lymphocytes ($p < 0.05$). An association between decreased switched-memory B cells with lymphoproliferation and malignancy was observed ($p < 0.03$ and $p < 0.05$, respectively). $CD4^+$ T-lymphocytopenia correlated with autoimmune phenotype, with 30% prevalence ($p < 0.05$). HLA-DR7 expression was related to CVID onset

Abbreviations: CD, celiac disease; CVID, common variable immunodeficiency; ESID, European Society for Immunodeficiencies; FI, fold increase; GI, gastrointestinal; HLA, human leukocyte antigen; IELs, intraepithelial lymphocytes; IgRT, immunoglobulin replacement; IIF, indirect immunofluorescence; IQR, interquartile range; IV, intravenous; MHC, major histocompatibility complex; NGS, next generation sequencing; nTreg, natural regulatory T-cells; PCR, polymerase chain reaction; PEG-IL2, polyethylene glycol-conjugated human recombinant interleukin-2; PIDD, primary immunodeficiency disorders; PPV, anti-pneumococcal polysaccharide; SC, subcutaneous; SD, standard deviation; SPSS, statistical product and service solutions; Treg, regulatory T-cells; TT, anti-tetanus antibodies; TV, anti-Salmonella antibodies; VEGF, vascular endothelial growth factor

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in early life in our patients (13 vs 25 years), and DQ2.5 or DQ2.2 with unexplained enteropathy ($p < 0.05$).
Conclusions: The phenotypic and genetic study is crucial for an adequate clinical orientation of CVID patients. In these two independent cohorts of patients, classification based in clinical and laboratory algorithms, provides more personalized treatment and follow-up strategies.

1. Introduction

Common variable immunodeficiency (CVID) is one of the most commonly symptomatic and heterogenous primary immunodeficiency disorders (PIDD) [2], with a prevalence of 1 in 25,000–50,000 individuals. Both genders are equally affected [3,4]. With the aim of better ranking the inherent heterogeneity of immunological features of CVID, the World Health Organization and the European Society for Immunodeficiencies (ESID) released the first consensus statements about the diagnostic criteria of CVID through a panel of experts [5].

The EUROclass trial was designed to define subgroups of CVID according to memory B cell phenotype, concealing the previous classification schemes of Paris and Freiburg, respectively [6]. However, this new classification did not encompass all the manifestations associated with the disease. Several attempts to refine this characterization have been made in the last years. In 2009, the International Union of Immunological Societies Expert Primary Immunodeficiency Committee redefined this condition as a "syndrome", given the broader range of clinical and laboratory findings, using the term “common variable immunodeficiency disorders” [7]. More recently, world CVID experts as Charlotte Cunningham–Rundles proposed that the diagnosis of CVID should be firstly based on clinical manifestations and established laboratory criteria and also considering novels biomarkers and genetic profiles [8,13]. She proposed a score with laboratory and clinical parameters for therapeutic decision-making [9], innovative scope further s validated by several groups [10–14]. Chapel et al. proposed in 2008 a classification based on CVID clinical phenotypes, which was subsequently revised, in 2012 [1]. This revised classification defines four main clinical phenotypes, which comprises cytopenia; polyclonal lymphocytic infiltration; unexplained enteropathy; and no disease-related complications. The present study aims to validate the classification of CVID according to the recently updated clinical and laboratory algorithms, based on different patterns of combinations of new CVID biomarkers, which could help to provide a more personalized treatment and follow-up strategies for the individual patient.

2. Methods

2.1. Design of the study

We retrospectively analysed a total of 80 CVID patients, 58 patients followed at the Department of Clinical Immunology of the Centro Hospitalar do Porto, Portugal, from now henceforth designated as "Portuguese Cohort"; and 22 CVID patients from the Department of Clinical Immunology at the Hospital Clínico San Carlos, Madrid, Spain, designated as "Spanish Cohort". The designation of the cohort implies only the centre where the patient is followed and not the origin of each patient. The data were analysed both comparatively between both cohorts and in a unified way.

Delay in diagnosis was defined as the difference between the estimated date of the onset of symptoms and date of established diagnosis, both reviewed in the clinical history of each patient.

2.2. Immunological assessment of CVID patients

Most of the procedures described below were performed routinely in the evaluation of patients with suspected CVID in both of the participating centres.

1 Humoral response (immunoglobulins and production of specific antibodies): Humoral responses were evaluated from serum samples of patients at diagnosis. Serum levels of IgG, IgA, IgM and IgE were studied by turbidimetry (The BindingSite LTD, Birmingham, United Kingdom) and ELISA (IgE). Reported immunoglobulins concentrations included into the analysis were measured before patients were started on immunoglobulin replacement therapy (IgRT). The levels of anti-pneumococcal polysaccharide (PPV) and anti-tetanus antibodies (TT) (*Portuguese cohort); and PPV, anti-*Salmonella typhi* Vi (TV) and TT (Spanish cohort), were quantified using commercial ELISA kits; anti-pneumococcal capsular polysaccharide IgG, VaccZyme™ human anti-*Salmonella Typhi* Vi IgG TV IgG, and tetanus toxoid IgG ELISAs (The Binding Site Group Ltd, Birmingham, UK), according to the manufacturer’s instructions. The following cut-off levels were used: pre PPV 27 mg/L as the approximate highest concentration of pneumococcal conjugated antibodies obtained in the vaccination schedule in a healthy population; IgG pre TV vaccination 28 U/mL (upper normal limit for pre-vaccination TV IgG concentration) [15,16]; and pre TT IgG 1 U/mL and [15,17]. Fold increase in concentration (FI) was determined using the formula (post-vaccination/prevaccination concentration). Responders were considered to achieve a FI of 3 for TV and TT IgG and 4 for PPV IgG, respectively [18,19].
2 Cellular evaluation: $CD4^+$ and $CD8^+$ T-lymphocytes, B lymphocytes and NK proportions and absolute counts of subsets of circulating immune cells were measured by multiparametric flow-cytometry, using a panel of specific markers. Functional characterization of T-cell subsets were studied by expression of $CD45RA^+$, $CD45RO^+$, $HLA\text{-}DR^+$, $CD25^+$, $FoxP3^+$.
3 Memory B cell phenotype was assessed by multiparametric flow-cytometry following EUROclass criteria. In male patients who presented < 2% of B cell, the BTK mutation/protein was performed to exclude X-linked agammaglobulinemia.
4 Analysis data of previous virological and bacteriological studies performed on patients with suspected active infection as per routine assessment were registered in the database.
5 For the evaluation of peripheral blood cytokine secretion, a panel of cytokines/chemokines were measured by Bio-Plex Pro™ human cytokine 27-plex kit (Bio-Plex) (#m500kcaf0y), from BioRad Inc by bead-based multiplex immunoassay. The sera were stored frozen at −70 °C before analysis. The panel includes IL-1B, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, basic FGF, eotaxin, G-CSF, GM-CSF, IFN G, IP-10, MCP-1 (MCAF), MIP-1alfa, MIP-1Beta, PDGF-BB, RANTES, TNF-alfa and VEGF. This test was performed in the patients of the Spanish cohort after obtaining informed consent for the study.

2.3. Genetic study

Human leukocyte antigen class II, (HLA)-DR, HLA-DQA1 and HLA-DQB1 loci detection were performed to the Spanish cohort. DNA was isolated from peripheral blood leukocytes, amplified by polymerase chain reaction (PCR) and hybridized according to standard techniques. Next Generation Sequencing (NGS) gene panels (within a library of 300 genes) was applied in patients who presented with severe manifestations or clinical concern in both cohorts. Specific cases required whole exome sequencing following laboratory-specific methodologies.

2.4. Statistical analysis

Data were entered in a Case Record Form using Excel. Categorical variables were summarized with absolute numbers and percentage and analysed with X2 test or Fisher's exact test when indicated. Continuous variables were represented with means and standard deviation (SD) or medians and interquartile range (IQR) depending on their homogeneity and will be compared using t-Student test, ANOVA, Mann-Whitney *U* test or Kruskall–Wallis test when appropriate. Multivariate logistic regression analysis was performed to identify predictive and/or prognostic factors. Variables with a p-value of ≤ 0.1 in bivariate analysis were identified and entered into the logistic regression model. All statistical tests were two-tailed, and a p-value of < 0.05 was considered to be statistically significant. Statistical analyses were performed using Statistical Product and Service Solutions (SPSS) software version 20.

2.5. Ethics

The study was approved by the centre's ethics committee of both Centro Hospitalar do Porto and Hospital Clínico San Carlos. Written informed consent of Hospital Clinico San Carlos was obtained in accordance to the Declaration of Helsinki. Exception of written informed consent of Centro Hospitalar do Porto was approved for the center's ethics committee, due to the characteristics of the study.

3. Results

3.1. Patients characteristics and immunophenotyping

The Portuguese cohort comprised 58 patients, with gender distribution of F:M 1:1. The median age at diagnosis was 33 years (range, 3–65). The majority of patients presented clinical onset during adulthood, although 24% debuted during childhood. The Spanish cohort consisted of 22 patients, with gender distribution of F:M 3:1. The median age at diagnosis of CVID was 31 years (range, 3–65). Three patients (14%) were diagnosed during childhood. In both cohorts, a mean-time of delayed diagnosis of 6 years was observed. We did not find any correlation between the diagnostic delay and baseline IgG levels.

In all patients, the measurement of specific antibody production was made prior to the introduction of IgRT. We found that 61% of the Portuguese cohort showed lack of specific antibodies responses. A third of the patients (32.56%) showed poor response for both protein and polysaccharides antibodies, 14% showed a lack of response to polysaccharide antibodies exclusively and equal proportion only to protein antibodies. The remaining (39%) of patients presented with recurrent infections, hypogammaglobulinemia, as well as other immunological alterations (suggestive of associated immunodeficiency), with normal antibody responses.

The Spanish cohort showed lack of specific antibodies responses in 93% of patients. Around 53% showed a lack of response for both protein and polysaccharide antibodies, while 40% evidenced a lack of response to polysaccharides exclusively. No patient in this cohort showed an exclusive defect against protein antibodies. Additionally, we evaluated the antibodies to common exanthematous viruses in the Spanish cohort. The majority of patients (n = 21/22), presented positive IgG against these microorganisms/germs. These data were not available in the Portuguese cohort.

In both cohorts of patients, IgRT was initiated except for two patients in the Spanish cohort. The score for IgRT [9], was used in treatment decision on the Spanish cohort. In the Portuguese cohort, the decision to IgRT was taken by different specialists. Based on laboratory and clinical parameters according to this scoring system, we found in the Spanish cohort a median laboratory score of 15, and a clinical score of 20, resulting in a cumulative score of 35.

All patients were monitored and maintained serum trough IgG levels ranging from $7-10$ g/L, with adequate tolerance to treatment. Mild adverse effects derived from the IV IgRT were reported (4% Portuguese Cohort *vs* 14% Spanish Cohort). Patients with adverse events required a switch to different presentations, such as SCIg of facilitated SCIg in weekly or monthly doses. IgRT product choice assigned to each patient depended on hospital availability and took into account the personal preferences of each patient.

IgA and IgM values were significantly low (at least 2 SD below the mean for age), more markedly for IgA. IgE was undetectable in 57% of patients, similarly to previous studies [20]. The findings in the humoral profile are similar in both cohorts.

3.1.1. Memory B cell phenotype

The median percentages of $CD19^+$ B lymphocytes did not differ between patients' cohorts. In the Portuguese cohort, we found that 55% of patients had increased naive B cells, as well as decreased marginal transitional cells and class-switched memory cells (63% and 71%, respectively), a trend similar to that observed in the Spanish cohort.

Following the EURO-Class classification of memory B phenotype, we found that most patients in the Portuguese cohort presented B^+ phenotype, while 9% (5/58) had a B^- phenotype. Nineteen percent were SmB- (switched memory B cell < 2%). In the Spanish cohort, 9% (2/22) had a B^- phenotype. Of patients with B^+ phenotype, 23% had SmB^-, with elevated expression of $CD21^{lo}$ plasmablasts in 40% patients.

3.1.2. Lymphocyte subsets and other plasma biomarkers

In the analysis of the lymphocyte subsets in the Portuguese cohort, we found normal percentages of $CD3^+$ T-lymphocytes in about half of the patients (31/58; 54%). Absolute counts were diminished in 42% (24/58). The Spanish cohort showed relative $CD4^+$ T-lymphocytopenia in more than half of the population (13/22, 59%). However, the absolute and percentage values of total $CD3^+$ as well as the $CD8^+$ T lymphocytes were within normal range. B lymphocytopenia (< 6% of total lymphocytes) was present in 36% (8/22) of the patients (9% with < 1% B cells). NK lymphocytes levels were expanded in about 10% in both cohorts.

In the Portuguese cohort, up-regulation of HLA-DR^+ was similar on $CD4^+$ and $CD8^+$ T-lymphocytes, predominantly in $CD8^+$ lymphocytes (60% *versus* 67%, respectively, $p < 0.05$), consistent with results from other cohorts [21,22]. Comparing the association of HLA-DR^+ with other T-lymphocyte activation markers, we found that increased HLA-DR^+ correlated with decreased $CD45RA^+$ expression in $CD8^+$ T-lymphocytes, while no correlation in $CD4^+$ was observed.

Serum levels of IL-5, IL-12, IL-10, IL-15 and vascular endothelial growth factor (VEGF) were undetectable in our patients of Spanish cohort. However, the expression of IL-1ra, IL-9, IP-10, RANTES, and PDGF-BB were increased compared to healthy controls (data not shown).

3.1.3. Genetics studies

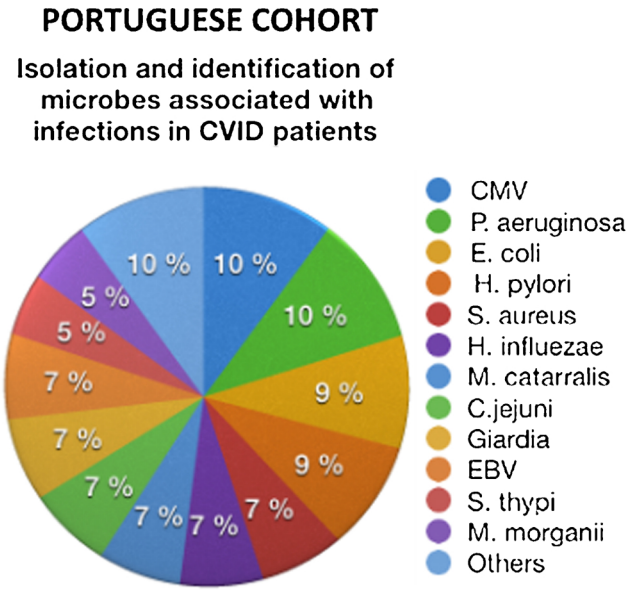
HLA class II in the Spanish cohort showed DR11 homozygosity in 2 patients (9%), originally from Romania and Spain, respectively. HLA-DR7 expression was related to t earlier onset of CVID in our patients (13 vs 25 years).

When comparing HLA class II expression between the Spanish population and CVID patients, we observed an increased frequency of DR4, DR7, DR11 and DQ5 ($p < 0.05$ all). The latter HLA expressions were related with autoimmune cytopenia, association previously described in non-CVID patients [23,24]. Ten out of 22 patients (45%) of the Spanish cohort presented gastrointestinal symptoms with diarrhoea resembling CD. Eighty (80%) of these patients carried HLA DQ2.5 or DQ2.2 ($p < 0.05$).

A genetic study based on NGS panel of genes related to CVID and whole exome sequencing was requested in 13% (10/80) of patients. Each case is briefly presented below.

- A 54-years-old male with panhypogammaglobulinemia, CD4 T-lymphopenia and decreased response to mitogens in CD4 and CD8 T-cells. He presented cytopenia and intestinal polyclonal lymphocytic infiltration. During follow-up progressive CD8 T-lymphocytosis (96% of total lymphocytes) associated to marked decrease in CD4 T-cells, B-cells and NK cells was observed. A genetic study and subsequent analysis by capillary electrophoresis of the amplified sequences were performed, observing the presence of clonal lymphocytes with rearrangement of the gamma-TCR, with suspected of T-LGL lymphoproliferative syndrome. The IDP genetic panel detected a mutation in PLCG2 and LRBA (both in heterozygosis). Both mutations would be likely considered disease-causing based on the mode of inheritance and/or patient phenotype. Homozygous mutations in LRBA have previously been associated with severe CVID phenotypes [25–27].
- A 31 years-old female patient with epilepsy since 18 months of age, recurrent upper and lower respiratory infections from 3 years of life, characteristic facies (goblin face), delay in growth with severe weight loss during adolescence, with refractory chronic diarrhoea. At age 21 withdraw anticonvulsants, she presented persistent panhypogammaglobulinemia, with suspected diagnosis of CVID. Three years later she was diagnosed with Addison's disease (with negative antibodies). This patient also presented cytopenia and unexplained enteropathy. A PID genetic panel was carried out, with mutations in the genes VPS13B, AIRE, NLRP12, CHD7, NCF2 (all in heterozygosis). These mutations have not been previously described in relation to CVID. These findings require functional studies to justify the associated clinical phenotype.
- Two first-degree relatives of 43-23-years old with CVID diagnosis. Both presented similar immunological alterations, with c recurrent respiratory infections, and autoimmune cytopenias. IDP panel study disclosed the mutation c.310 T > C (p.Cys104Arg) and c.512 T > G (p.Leu171Arg in heterozygosis of the TNFRSF13B gene.
- Thirty-eight years old female patient with characteristic facies, short stature, intellectual disability, chronic unexplained enteropathy,

Isolated Microbes	N- (%)
CMV	6 (10)
P. aeruginosa	6 (10)
E. coli	5 (9)
H. pylori	5 (9)
S. aureus	4 (7)
H. influezae	4 (7)
M. catarralis	4 (7)
C. jejuni	4 (7)
Giardia	4 (7)
EBV	4 (7)
S. typhi	3 (5)
M. morganii	3 (5)
Others	6 (10)



Isolated Microbes	N- (%)
CMV	5 (20)
H. influezae	4 (15)
EBV	3 (12)
Herpes simplex	3 (12)
H. pylori	2 (10)
Rhinovirus	1 (7)
Giardia	1 (7)
C. jejuni	1 (7)
Others	2 (10)

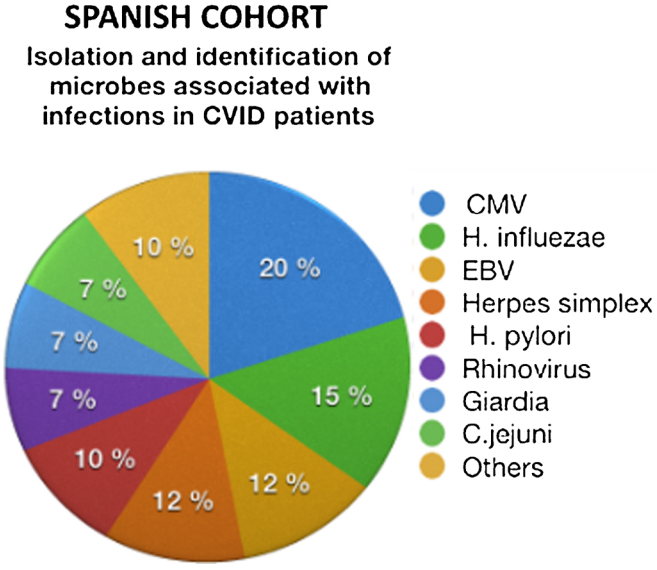


Fig. 1. Infectious manifestations in CVID.

Table 1
CVID Clinical manifestations and phenotypes. A. Comparison of infectious manifestations in both cohorts. B. Non-Infectious manifestations (Clinical Phenotyping) based in classification proposed by Chapel et al.

A. Infectious manifestations	Portuguese cohort N- (%)	Spanish cohort N- (%)
Upper respiratory infections	27 (47%)	13 (60%)
Lower respiratory infections	28 (48%)	11 (50%)
Gastrointestinal infections	11 (19%)	0 (0%)
Dermatological infections	8 (14%)	2 (9%)

B. Non-infectious manifestations (Clinical phenotyping)	Portuguese cohort N = 58- (%)	Spanish cohort N = 22- (%)	Total N = 80
Cytopenia	25 (43%)	7 (32%)	32 (40%)
Polyclonal lymphocytic infiltration	11 (19%)	4 (18%)	15 (19%)
Unexplained enteropathy	8 (14%)	2 (9%)	10 (12%)
No disease-related complication	3 (5%)	1 (5%)	4 (5%)
Overlap	11 (19%)	8 (36%)	19 (24%)

severe refractory ITP, hypothyroidism, and immunological profile of CVID Whole exome sequencing study showed heterozygosity for the mutant allele p.Trp268Cysfs *36 in exon 6 of the KMT2D gene, confirming Kabuki syndrome (KS). Mutations in KMT2D and KDM6A have been identified as the main cause for KS. Similar to CVID, patients with this syndrome have recurrent infections, reduced immunoglobulins levels and autoimmunity [28,29].

- A 26-years-old female patient with clinical onset in childhood with serious recurrent infections, like severe ulcerative CMV enterocolitis and salmonella coinfection, secondary malnutrition, CMV endometritis, lung tuberculosis, esophagitis, EBV-associated lymphoproliferation. The immunological profile showed a marked lymphocytopenia B, with $CD4^+$ and $CD8^+$ T lymphocytes in the lower limit of normal range, very low mitogen-lymphoproliferative responses t. Patient presented cytopenia (PTI) and polyclonal lymphocytic infiltration (polyadenopathies). A whole exome sequencing did not fin a causative mutation. Due to severe clinical phenotype and lack of response to several treatment lines, hematopoietic stem cell transplant was decided as a therapeutic measure.
- Tweenty-two years old male patient, with family history of consanguinity and childhood death of his sister due to infection suspected PID. He presented with recurrent oral aphtha, recurrent and severe cutaneous and upper and lower respiratory infections, secondary septic shock. The patient developed a diffuse non-Hodgkin B lymphoma stage II and received chemo and radiotherapy. NGS genetic study did not find a causative mutation. Due to relapse of the lymphoma the patient died.
- Male patient with recurrent upper respiratory infections and urinary tract infections since childhood, one episode of bacteraemia due to S. epidermidis. Hypogammaglobulinemia and specific antibodies deficiency deficit were found. At age 12, he presented Hodgkin lymphoma stage IV-L, treated with chemo and radiotherapy, with complete remission until age 18, when he suffer a recurrence. He also presented cytopenias and unexplained enteropathy. Whole exome sequencing disclosed homozygous PLCG2 and TACI (TNFRSF13B), and heterozygosis in LRBA mutations.
- Two patients of forty and forty-six years old, respectively, one of them with history of ITP in childhood and upper and lower recurrent respiratory infections, with i CVID type B-, and the other one with systemic granulomatous disease, recurrent infections and CVID profile with T cellular alterations. NGS panel for CVID was performed, without any findings.

3.2. Clinical manifestations

The most frequent infections reported in the Portuguese cohort was recurrent upper and lower respiratory tract infections (48% and 47%, respectively), as well as the gastrointestinal tract infections (19%), with diarrhoea of difficult management. Dermatologic infections, such as skin abscesses ranked third in this cohort, followed by genitourinary infections. Nine out of fifty-eight (16%) of the patients had a severe infection that required intermediate or intensive care hospitalization.

The Spanish cohort presented a similar distribution of infections, with the respiratory tract being the most frequently affected, where upper and lower infections presented a similar casuistry (60% and 50%, respectively). Gastrointestinal, genitourinary and dermatologic infections had a lower prevalence. In most of the patients of this cohort who presented acute and self-limited diarrhoea, no micro-organism was identified. A small proportion of patients presented recurrent infections as the only CVID manifestation. A 5% (n = 3) of the Portuguese cohort and 5% (n = 1) of the Spanish cohort, associated exclusive respiratory tract compromise.

The microorganisms most frequently isolated when cultures were performed in the Portuguese cohort were *cytomegalovirus* (CMV) and *P. aeruginosa* in the first place, followed by *Escherichia coli and H. pylori.* Other agents with important prevalence include *Staphylococcus aureus, Haemophilus influenzae, Moraxella catarrhalis, Campylobacter jejuni, Giardia*, *Epstein–Barr virus* (EBV). All the patients with identification of *P. aeruginosa* (n = 6/6) and *E. coli* (n = 3/5) from the sputum showed established lung damage mainly bronchiectasis. Two patients with sputum isolation of *E. coli* presented recurrent urinary tract infections.

In the Spanish cohort, the germs most frequently isolated were CMV and *H. influenzae*, with a similar distribution in the isolation of microorganisms such as viruses and parasites, when compared to the Portuguese cohort (Fig. 1). CMV and EBV infections were confirmed by virus PCR in both cohorts.

In most cases of infection-related symptoms, the agent involved was not known, due to empirically antibiotic therapy.

With respect to non-infectious clinical manifestations in the Portuguese cohort, splenomegaly and autoimmune diseases (mainly immune cytopenia) were the most common clinical features (Table 1). Important to note the frequency to immune thrombocytopenia purpura (ITP) in these patients (16%). These two manifestations were the most frequently observed in the Spanish cohort as well. However, splenomegaly was found in up to half of the patients, while autoimmunity was present in a third of them.

The incidence of splenomegaly was 38% in the Portuguese cohort and 50% in the Spanish cohort, respectively ($p \geq 0.05$). Most published series indicate an average incidence around 30% [30,31]. Several cases of the Portuguese cohort underwent splenectomy in the context of ITP (16%), while none of the Spanish cohort.

We categorized our cohort of patients based on the revised classification of clinical phenotyping proposed by Chapel et al. [1]. We found that 76% of patients entered within one of the four categories (cytopenia; polyclonal lymphocytic infiltration; unexplained enteropathy; and no disease-related complications). The remaining 24% showed overlap between phenotypes. The most prominent phenotype was “cytopenia” (40%) followed by “polyclonal lymphocytic infiltration” (19%). The remaining 24% of our cohort had overlap of 2 clinical phenotypes (cytopenia and unexplained enteropathy mainly).

3.2.1. Cytopenia and organ-specific autoimmune diseases

Forty out of 58 patients (69%) from the Portuguese cohort presented autoimmunity, of which 33 out of 58 (57%), presented peripheral cytopenia (mainly ITP), with 30% of bi- or pancytopenia (Evan's syndrome), affecting the red series and platelets in a similar proportion. Leukocytopenia was present in 48%. Only two cases (3%) of eosinophilia were reported. About 64% (14/22) of the Spanish cohort presented autoimmunity, the most common 59% (13/22) was peripheral

cytopenia. These patients associated Evans syndrome in 45%, mainly in red cells and platelets. The decrease of a single cell line showed a similar distribution in both groups. Twenty five out of 58 (43%) of the Portuguese cohort and 7 out of 22 (32%) of the Spanish cohort presents exclusively peripheral cytopenia as a autoimmune manifestation.

Other autoimmune manifestations in both cohorts in very low proportions included autoimmune thyroiditis (n = 2), systemic lupus erythematosus (SLE) (n = 1), Raynaud's syndrome (n = 1), autoimmune gastritis (n = 1), systemic sclerosis (n = 1), and mixed connective tissue disease (n = 1) with positive auto-antibodies tested. In our cohorts, males and females appear to have autoimmunity in equal numbers, in contrast to non-PID populations.

3.2.2. Polyclonal lymphocytic infiltration and malignancy

In our study, six patients (8%) presented granulomas, 4 patients of Portuguese cohort (7%) and 2 patients of the Spanish cohort (9%). Three of them had granulomas in lymph nodes, two in the liver and one patient had systemic granulomatous disease (disseminated granulomas in various organs including lungs, liver, and brain). Seven out of 58 (12%) of the Portuguese cohort and 2 out of 22 (9%) of the Spanish cohort presented lymphoproliferative disease without malignancy.

In the Portuguese cohort, malignancy was reported in 21% (11/58) of CVID patients, firstly, haematological malignancy, mainly lymphomas (12%). Of these, non-Hodgkin B-cell lymphomas were the most common. The incidence of solid tumour was 9%, related to GI cancer in the first place (stomach, colon and rectum), followed by genitourinary and endocrine cancer. In the Spanish cohort, 23% (5/22), presented diverse malignancy (Hodgkin's lymphoma, ovarian cancer, thyroid cancer and pancreas cancer).

Thirteen percent of our patients with a history of malignancy had B-cells lymphocytopenia (< 5% of total lymphocytes), nine out of 14 (64%) of the Portuguese cohort and 1 out of 4 of Spanish cohort (25%), respectively.

3.2.3. Unexplained enteropathy

In 17% of the Portuguese cohort and 45% of the Spanish cohort, chronic diarrhoea was present (without microorganism identified). Eighty percent (8/10) of the Spanish cohort with gastrointestinal (GI) manifestations presented celiac-associated HLA markers without autoantibody detection ($p < 0.05$). In patients with non-infectious GI manifestations, antibodies against celiac disease (anti-tissue transglutaminase antibody and anti-deamidated gliadin peptides) were tested, all of them were negative.

Eight out of 58 (14%) of the Portuguese cohort and 2 out of 22 (9%) of the Spanish cohort presented with sole unexplained enteropathy as clinical manifestation, whereas the rest of the patients presented overlapping of enteropathy with another phenotype (cytopenia mainly).

4. Discussion

CVID is one of the most commonly symptomatic and heterogeneous primary immunodeficiency disorders (PIDD) [2]. We found a slight higher prevalence in the female gender in the Spanish cohort in relation to the Portuguese cohort, with a similar median age in both at diagnosis. Although women tend to manifest later in life, CVID has no clear gender preference and in contrast with normal population, the survival rate is similar for both sexes [32,33]. A delay of 6 years in CVID diagnosis is similar to that observed by other authors [12,14,34]. We assume that this result may be influenced by the wide age range in the patients' population and hence the old date of diagnosis. Delayed diagnosis will tend to decrease as the knowledge and diagnostic criteria of this pathology improves.

More commonly used vaccines for testing antibodies responses in CVID patients are DTP (diphtheria, tetanus and pertussis vaccine), conjugated pneumococcal vaccine followed by polysaccharide vaccine and influenza vaccine. Unresponsiveness to vaccination is considered as diagnostic criteria for CVID [2,35,36]. Antibodies´ production should be interpreted together with specific baseline levels. Circulating concentrations of pneumococcal antibodies due to immunization or previous endemic exposure to pneumococcus may be found in PIDDs, make it difficult to interpret with pneumococcal polysaccharide vaccine response, especially in countries where the measuring isolated serotypes against pneumococcus is not available [37]. The response to another pure polysaccharide vaccine like Typhim Vi IgG seems to be a useful accessory tool to determine specific antibody production this population [18,36,37].

The majority of patients of the Spanish cohort presented positive IgG against common childhood exanthematous viruses, which might reflect competence of antibody responses at the time of interaction with these pathogens (childhood) and maintenance of immune memory to them.

Most of the current guidelines support the use of intravenous (IV) or subcutaneous (SC) IgRT in symptomatic CVID patients as the mainstay of prevention against infections. Nevertheless, IgRT may be postponed in CVID patients with low rate of infections without clinical aftermath [9,38–40]. The score based on laboratory and clinical parameters is useful for therapeutic decision-making. Our results are similar to those by Cunningham–Rundles [9]. Adverse effects derived from the IV IgRT were less common than those reported in previous studies [41–43], and other administration options, such as a switch to presentations of SC or FSC were proposed.

Adequate $CD4^+$ T-lymphocyte-dependent B-cell maturation and differentiation involves appropriate co-stimulatory signals such as JAK/STAT activation [44]. We observed $CD4^+$ T-lymphocytopenia was associated with the presence of autoimmune disease included in cytopenia's phenotype, we found a 30% prevalence in both cohorts ($p < 0.05$), similarly to previous studies conducted by Chapel et al. [30]. $CD4^+$ T-lymphocytopenia was not associated with other clinical phenotypes in our cohorts.

While the hallmark of CVID is the B cell memorydefect, many patients also have variable T-cell abnormalities and cytokine defects (20% approximately) [45,46]. HLA-DR expression in T-lymphocytes is considered as a marker of their activation status with alterations linked to specific genotypes expression [47,48]. In our study, increased memory $CD45RO^+$ T-lymphocytes correlated with decreased switched memory B-cells ($p < 0.05$), which might suggest defective T-B cells co-stimulation. Expansion of memory $CD45RO^+$ T-lymphocytes with decreased naive $CD45RA^+$ cells population has been described in previous studies [21,49]. This memory/naive T cell distribution was observed in our cohort ($p < 0.05$). Persistent activation of T- cells might be influenced by subclinical infection and intestinal dysbiosis in patients with CVID [50,51]. The increased activation of T-lymphocytes was significantly related to clinical phenotypes, such as cytopenia (33%), lymphoid proliferation (27%) and/or malignancy (23%), respectively. Malignancy correlated with increased $CD8^+CD45RO^+$ T-lymphocytes ($p < 0.05$). In this regard, Yu et al. showed that $CD45RO^+$ regulatory T cells (T_{Reg}) were significantly increased and $CD45RA^+$ T-lymphocytes significantly lower in CVID subjects with autoimmune disease compared to patients without autoimmunity [52].

Regulatory $CD25^+FoxP3^+CD4^+$ T-lymphocytes (nT_{Reg}) are a subset with potent immunoregulatory roles [3], essential in down-regulation of the effector immune response and in maintaining self-tolerance [53]. In our cohort, we found heterogeneous increase of T_{reg} in 35% of patients while decreased nT_{Reg} in 26%. The suppressive function of nT_{Reg} was altered in CVID patients, which may contribute to autoimmune pathogenesis. However, no clear correlation was found between increased or decreased proportions of nT_{Reg} and autoimmune diseases including cytopenia in our cohort, but study of nT_{reg} deserves further investigation.

Several studies have identified abnormalities in peripheral cytokine secretion, including decreased production of IFN-gamma, IL-2, IL-5, IL-

7, IL 4, IL-10 or IL-12 in CVID patients. This might be due to genetic polymorphisms affecting these cytokine genes [54–59]. An imbalance between the regulatory and inflammatory response has been suggested to contribute to the dysregulated profile in the setting of immunodeficiency. When we compared with the expression of serum cytokines, a marked absence in the levels of IL-2 and IL-10 in all the patients was observed with respect to healthy controls. Several studies showed that both molecules are crucial for activation and homeostasis of lymphocytes, mainly T_{Reg} and in the differentiation of B lymphocytes [3,60–63]. Supporting this latter concept, PEG-IL2 treatment improved T cell helper activity in some patients [64], find association of cytokine expression with different clinical phenotypes.

Most CVID cases occur sporadically, but familial cases can also occur in up to 20%. A strong association with MHC, mostly an expression of genetic loci present in chromosomes 4q, 6p, and 16q, has been described [47,65]. Particular polymorphisms in genes as TACI (TNFRSF13B, MSH5, TWEAK, BAFFR), ICOS, PIK3CD, MAP3K7IP3, PFTK1, HAVCR1, KIAA0834, CACNA1C, LRBA, STAT3, STXBP2, ADAM28, ADAM7, ADAMDECI, SDK1, UBX10 are possibly involved in CVID pathogenesis [45,47,66].

HLA loci can exhibit a remarkable degree of allelic polymorphism, whose variability determines the immune response of an individual related to the presentation of peptides to T-lymphocytes, which explains the exteded variability in response to certain infectious agents [67]. Several studies have shown that the majority of patients with IgA deficiency and CVID share one of three extended MHC haplotypes marked by either HLA-DQ2, -DR17 and -B8; or HLA-DQ2, -DR7 and -B44 [65]. Presenting certain HLA antigens may confer susceptibility to specific diseases [67]. Our results confirm previously reported CVID association with DR4, DR7 haplotypes [65]. HLA-DR7 expression was related to the clinical onset CVID in early life, which warrants further validation.

Several studies have associated diverse relative risk according to the ethnic groupand the allelic expression of HLA-DR and HLA-DQ, with different autoimmune diseases, such as Addison's disease, celiac disease (CD), autoimmune thyroiditis, diabetes mellitus type I, myasthenia gravis, rheumatoid arthritis and SLE [67–69]. In our study, DR4, DR7, DR11 and DQ5 expression were related with autoimmune cytopenia, although without significance. This association has been previously described in non-CVID patients [23,24].

We observed an increased frequency of certain haplotypes related to the disease when comparing HLA class II expression between the Spanish population and CVID patients (Table 2). In fact, 80% of the Spanish cohort classified into the unexplained enteropathy phenotype presented HLA DQ2.5 or DQ2.2 expression ($p < 0.05$).

At least in CVID patients with severe phenotype, genetic sequencing should be considered in order to detect themutation responsible for the clinical presentation. The case review was carried out in search of highlighting any possible association between the clinical phenotypes involved and their genetic expression. There are tens of genes that have been described in association with the development of CVID accounting for up to 15% of CVID patients. However, a great heterogenicity of results (several of them inconclusive) in patients who had been classified as possible CVID remains. The overlap of clinical phenotypes should give an alert signal about the suspicion of specific mutations that could condition a more severe evolution of the disease. Genetic testing of a panel of CVID related genes is essential in the differential diagnosis and precision medicine approach. The integration of clinical, biological, immunological biomarkers, lifestyle and genetic defects conforms the basis of a desirable personalized healthcare. However, to elucidate the specific genes that may be involved, more studies with a broader cohort are needed. This underlines the relevance of a thorough study and the path ahead to better understand PIDs with CVID-like manifestations.

Over 90% of CVID patients suffer from an increased susceptibility to different pathogens affecting several systems [2,45,70]. The microorganism most frequently isolated was CMV in both cohorts (Fig. 1). To note, the prevalence of bacterial infections could be falsely decreased due to antibiotic prophylaxis in patients with specific conditions. Recurrent respiratory infections were the most frequent infections presented in both cohorts. These findings similar to previous studies [2,71]. Such recurrence could predispose the development of chronic lung disease, like bronchiectasis. Bronchiectasis represent a leading cause of both morbidity and mortality in CVID patients [13,30,72].

A low prevalence of patients who have presented as the only symptom recurrent infections in our cohorts was observed (5% in both Portuguese cohort and in Spanish cohort, respectively). These patients would be included in “no other disease-related complications”, according with clinical phenotype classification. These results contrast with that described in previous studies [1].

Besides infections, splenomegaly hypersplenism or uncontrolled autoimmunity was a common complication. The incidence varies in the population studied, splenomegaly was more prevalent in the Spanish cohort with respect to the Portuguese cohort (50% vs 38%, respectively). Although splenomegaly is very common in CVID patients, its role as a risk and prognostic marker is not entirely clear [30].

Approximately, 25% of CVID subjects develop autoimmune disease in published series [52,74,75]. The pathogenesis of autoreactivity is not completely understood, which target predominantly peripheral blood cells [75]. Indeed, ITP and autoimmune haemolytic anaemia are the most commonly reported [52,73,74]. Organ-specific autoimmunity was not associated with cytopenias or the other clinical phenotypes, we noted them for their clinical significance in the prognosis of our patients.

Table 2
Main HLA class II phenotype frequency in CVID patients in Spanish Cohort.

HLA class II	CVID N (%)	Spanish population	P
DR1	4 (18%)	0.107	0.0204
DR3	4 (18%)	0.134	0.034
DR4	7 (32%)	0.123	0.0014
DR7	8 (36%)	0.159	0.0016
DR8	2 (9%)	0.023	0.009
DR11	10 (45%)	0.109	< 0.001
DR13	3 (14%)	0.119	0.0496
DR14	1 (5%)	0.029	0.0477
DR16	1 (5%)	0.025	0.0427
DQA1*01:01:01	5 (23%)	0.084	0.0033
DQA1*01:02:01	1 (5%)	0.102	0.0328
DQA1*01:03:01	2 (9%)	0.073	0.0484
DQA1*02:01:01	8 (36%)	0.161	0.0005
DQA1*03:01:01	5 (23%)	0.071	0.0017
DQA1*03:02:01	2 (9%)	0.009	0.0017
DQA1*05:01:01	2 (9%)	0.053	0.0027
DQA1*05:01:02	4 (18%)	0.071	0.0469
DQA1*05:05:01	10 (45%)	0.134	0.0006
DQA1*06:01:01	2 (9%)	0	–
DQB1*02:01:01	7 (32%)	0.135	0.0066
DQB1*02:02:01	5 (23%)	0.144	0.0201
DQB1*03:01:01	13 (59%)	0.029	< 0.001
DQB1*03:01:03	1 (5%)	0.109	0.0291
DQB1*03:02:01	3 (14%)	0.092	0.033
DQB1*03:04:01	1 (5%)	0.003	0.0064
DQB1*04:02:01	1 (5%)	0.029	0.0477
DQB1*05:01:01	2 (9%)	0.024	0.0097
DQB1*05:01:05	3 (14%)	0	–
DQB1*05:02:01	1 (5%)	0.029	0.0477
DQB1*05:03:01	1 (5%)	0.027	0.0452
DQB1*06:03:01	3 (14%)	0.078	0.0244

Extracted data: G. Montero-Martín, K.C. Mallempati, S. Gangavarapu, F. Sánchez-Gordo, M.J. Herrero-Mata, A. Balas, J.L. Vicario, F. Sánchez-García, M.F. González-Escribano, M. Muro, M.R. Moya-Quiles, R. González-Fernández, J.G. Ocejo-Vinyals, L. Marín, L.E. Creary, K. Osoegawa, T. Vayntrub, J.L. Caro-Oleas, C. Vilches, D. Planelles, M.A. Fernández-Viña, High-resolution characterization of allelic and haplotypic HLA frequency distribution in a Spanish population using high-throughput next-generation sequencing, Hum. Immunol. 80 (2019) 429–436. doi:https://doi.org/10.1016/j.humimm.2019.02.005.

Cytopenias was the third most prevalent phenotype after no disease-related complications and polyclonal lymphocytic infiltration in the cohort studied by Chapel et al. [1]. In our study, cytopenias was the most frequent clinical phenotype in both cohorts. Evan's syndrome and ITP were the main manifestations.

We found a relationship with SmB- and autoimmune disease including cytopenias ($p < 00.5$). Several reports have shown the reduced amounts of SmB- ($CD27^+IgD^-IgM^-$) in CVID [2,11,34,57]. Patients with SmB- cells subset, in special with elevated expression of $CD21^{lo}$ plasmablasts, was considered with elevated independent risk factor of develop autoimmune disease [76,77].

The activation of distinct immune cells and release of diverse cytokines due chronic inflammation may trigger the development of autoimmune disease in genetically susceptible individuals. [74,75,78]. The pathophysiology of the processes involved remains undefined and is heterogeneous, nevertheless, it is considered to be related to B- and T-cell dysfunction [8,74,75]. The persistent cellular activation and chronic inflammation, with tissue destruction, might result in the formation of anti-tissue antibodies even previously to the presentation of clinical symptoms of the disease.

According to the classification, polyclonal lymphocytic infiltration includes polyclonal lymphoproliferation, persistent unexplained lymphadenopathy, lymphoid interstitial pneumonitis, and noninfective granuloma, which will be discussed below. We will also discuss the presence of malignancy in our cohort for the close relationship with this clinical phenotype.

Granulomatous disease may be a clinical sign of immunodeficiency, observed in 8%–22% of CVID patients [79,80]. This condition could be associated with higher incidence of autoimmunity and shorter survival [81]. However, this manifestation is unusual CVID complication, the prevalence of granulomatous disease may vary according to the population studied [30,79]. In most cases, granulomatous lesions are found in the lung, lymph nodes, liver and skin [79,80], as well as in several organs simultaneously and have very varied clinical presentations suggesting an unusual form of an inflammatory reaction [82]. We found no association of autoimmunity with granulomatous disease, probably due to small sample size.

Polyclonal lymphocytic infiltration was the second phenotype most prevalent in our cohort. The patients with this clinical phenotype must have a close medical follow-up due an increased risk of developing malignancy. The polyclonal lymphocytic infiltration was associated with 8-fold increased risk for females and 10-fold increased risk for males of lymphoid malignancy, according to previously published studies [30,83].

The increased risk of cancer in CVID may result, among other factors, from impaired immunity to potentially carcinogenic pathogens [84]. Immune surveillance seems crucial for the control of certain cancers, whereas its defect could condition the development and extension of malignancy. CVID patients presented an increased global incidence of malignancies, especially lymphomas [13,45]. In published series, the prevalence of GI cancer is not clear [85]. This cancer may be related to lymphoproliferation within the digestive tract as well as to persistence of microbial infections.

The long-term outcome was significantly influenced by a delay of CVID diagnosis and treatment, conditioned by the presence of chronic inflammatory complications [85,86]. In our study, patients with more than 6 years of delay of diagnosis, presented 3.7-fold higher risk of developing lymphoproliferation and/or malignancy ($p < 0.05$). This aspect requires a deep study and highlights the relevance of an early CVID diagnosis.

We found a relationship between B-cells lymphocytopenia, history of malignancy and polyclonal lymphocytic infiltration ($p < 0.05$), compatible with previous studies [6,76,83,87]. The pathophysiological relationship between these changes and malignancy is not understood [85]. In fact, SmB- CVID was an independent risk factor of lymphoid proliferation, autoimmune disease and splenomegaly [76].

The prevalence of GI complications was high in CVID patients [88]. Persistent diarrhoea has been reported in more than 50% of CVID cases in the literature [89]. Our study showed overlapping between unexplained enteropathy and cytopenia's in phenotype score mainly, but not a clear relationship with $CD21^{lo}$ B-cells, finding that has been described in other studies [89,90].

A large percentage of patients in the Spanish cohort presented chronic diarrhoea, suggesting inflammatory enteropathy, with celiac-associated HLA markers but without autoantibody detection ($p < 0.05$). Ten out of 22 CVID patients (45%) of the Spanish cohort presented CD-like GI symptoms. Eight (80%) of them carried CD-associated HLA DQ2.5 or DQ2.2. Interestingly, HLA DQ2.5 or DQ2.2 was significantly higher in CVID with GI symptoms compared to CVID without GI symptoms ($n = 2$ out of 12, 16%, $p = 0.003$) and to Caucasian population carriers (20% vs–40%, $p < 0.0001$) [89–92]. HLA-class II predisposition is necessary but not sufficient for development of CD. Therefore, the absence of these alleles has a high negative predictive value for CD [88,90]. A deeper study through intraepithelial lymphocytes (IELs) phenotyping in CVID patients with GI manifestations and genetic predisposition with negative celiac autoantibodies may shed light onto the diagnosis, despite the biopsy specificity is not entirely clear in these patients [89]. In any case, the recommended treatment would be gluten-free diet and strict follow-up. If patients test negative for CD-associated HLA-DQ markers or there is no improvement after the gluten-free diet, after aggressive search for infectious agents an autoimmune enteropathy needs to be also considered and may be treated by immunosuppressive therapy. In this context, the determination of norovirus type 2 was not routinely requested in our cohorts [93].

There are not standardized therapeutic guidelines or clinical trials for the use of immunosuppressive and immunomodulatory agents in different clinical phenotypes expression of CVID. Corticosteroids are considered the first line therapy for the autoimmune and inflammatory manifestations (cytopenias, polyclonal lymphoproliferation and unexplained enteropathy) [74,75]. Nevertheless, control of these clinical manifestations often requires different lines of treatment, such as hydroxychloroquine, cyclosporine, methotrexate, rituximab, TNF-alpha antagonists, chemotherapeutic agents or hematopoietic stem cell transplant [94,95], which may further induce immunosuppression in these patients.

The development of granulomatous disease, autoimmunity or lymphoproliferation associated to haematological malignancy in CVID patients is of serious concern. Patients that require a variety of immune suppression treatments could develop more severe kind of immunodeficiency [94]. Immunosuppressive agents must be used with caution in CVID patients. Low dose corticosteroids are often helpful in controlling some clinical manifestations, nevertheless, long periods of treatment are not recommended.

5. Conclusions

We believe that the detailed phenotyping of these two geographically distinct cohorts replicates other previous studies and highlights that CVID classification based on clinical and immunological parameters is still valid. Genetic testing of a panel of CVID related genes is essential in the differential diagnosis and precision medicine approach. The integration of clinical, biological, immunological biomarkers, lifestyle and genetic defects conforms the basis of a desirable personalized healthcare. Phenotypic and functional studies and recent advances on genetic diagnosis convey on different biomarkers in CVID patients helping to predict the spectrum of complications and progression of the syndrome. The delay in the diagnosis could condition the development of associated complications and the quality of life of the patient. Hence, reducing diagnostic delay is crucial and requires education and awareness of clinicians at different levels of care.

The clinical manifestations of CVID are widely heterogeneous and

relate with underlying immune defects [11]. The landscape encompass recurrent infections but also characteristic inflammatory or autoimmune conditions, often before infections occur [74]. Failures in the regulatory mechanisms of self-tolerance could be responsible for the development of autoimmune and inflammatory disease. Laboratory biomarkers might be useful tools to stratify risk for complications and survival in these patients. In progress toward a molecular understanding of CVID, a small number of specific single gene disorders have been discovered, while these do not explain the majority of CVID cases. We consider that the genetic study is essential in CVID patients.

In summary, an adequate differential diagnosis and the phenotypic and genetic study of these patients is crucial for an adequate clinical orientation. In this context, a classification based in clinical and laboratory algorithms, that encompasses the immunological phenotype as well as the clinical profile help to provide more personalized treatment and follow-up strategies. We must deepen knowledge in this syndrome, in the search for new biomarkers to predict risk and forecast in CVID continues,however it is a difficult task in such a heterogeneous disease.

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Declaration of Competing Interest

The authors declare no other competing financial interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.imlet.2020.03.006.

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Glossary

Recurrent infections: ≥3 infections in one year, ≥ 2 severe infections in one year, or the need for antibiotics for two months/year [96]

Severe/serious infections: include persistent fever or confinement to bed for a week or more, failure to respond to oral antibiotics and/or the need for intravenous antibiotics or hospitalization, infections with an unusual pathogen, unusual complications (eg mastoiditis, pleural effusion, abscesses), or persistent laboratory abnormalities [96]

Laboratory reference: values including immunoglobulin levels and lymphocyte immunophenotype were obtained from published guidelines The normal ranges of these parameters were adjusted according to age group, following parameters previously published [97–100]

Celiac autoantibodies tested: IgA anti-tissue transglutaminase, IgG anti-deamidated gliadin peptide and anti-endomysium

Naïve CD45RA$^+$ T-cells: (CD3$^+$ CD4$^+$/CD8$^+$ CD45RA$^+$ CD45RO − CCR7$^+$ CD27$^+$)

Memory CD45RO$^+$ T-cells: (CD3$^+$ CD4$^+$/CD8$^+$ CD45RA- CD45RO$^+$ CCR7$^+$ CD27$^+$)

HLA class II: HLA-DQ2.5 encoded by DQA1 *05:01 and DQB1 *02:01, HLA-DQ2.2 encoded by DQA1*02:01 and DQB1 *02:02, HLA-DQ8 encoded by DQA1 *03:01 and DQB1 *03:02, HLA-DQ5 encoded by DQA1*01, DQB1 *05. HLA-DQ7 encoded DQA1*05, DQB1 *03

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Serum Free Immunoglobulins Light Chains: A Common Feature of Common Variable Immunodeficiency?

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Serum free light chain (sFLC) is a recently proposed biomarker for CVID diagnosis. Most CVID patients present low or undetectable sFLC up to 10-fold lower compared to other primary antibody deficiencies. Given that κ and λ light chains are normally secreted in excess with respect to immunoglobulins, this finding points to an intrinsic defect of B cell differentiation in CVID. sFLC levels were prospectively evaluated in a cohort of 100 primary immunodeficiency (PID) patients and in 49 patients with secondary immunodeficiency to haematological malignancy (SID). CVID patients had significantly lower κ and/or λ values (mean: κ: 1.39 ± 1.7 mg/L and λ: 1.97 ± 2.24 mg/L) compared to "other PIDs" (κ: 13.97 ± 5.88 mg/L and λ: 12.92 ± 7.4 mg/L, respectively, $p < 0.001$ both), and SID (κ 20.9 ± 22.8 mg/L and λ 12.8 ± 8.7 mg/L, respectively, $p < 0.001$ both). The sum of kappa and lambda (sum κ + λ) in CVID patients (7.25 ± 7.90 mg/L) was significantly lower respect to other PIDs (26.44 ± 13.25 mg/L, $p < 0.0001$), and to SID patients (28.25 ± 26.24 mg/L, $p = 0.0002$). ROC analysis of the sum κ + λ disclosed an area under the curve (AUC) of 0.894 for CVID diagnosis (SD 0.031; 95% CI: 0.83–0.95, $p < 0.0001$), with optimal cut-off of 16.7 mg/L, giving the highest combination of sensitivity (92%), specificity (75%) and NPV (98%). The Relative Risk (RR) for patients presenting a sum κ + λ below 16.7 mg/L was 20.35-fold higher (95%, CI: 5.630–75.93) for CVID than below this threshold. A similar behavior of the sFLC in our CVID cohort with respect to previously published studies was observed. We propose a cut-off of sum κ + λ 16.7 with diagnostic application in CVID patients, and discuss potential specific defects converging in low or undetectable sFLC.

Keywords: common variable immunodeficiency, serum-free immunoglobulins light chains, diagnostic tool, prognostic biomarkers, primary immunodeficiencies

BACKGROUND: IMMUNOGLOBULIN, THE MASTER KEY OF MANY LOCKS

Given the high clinical variability and immunological heterogeneity in clinical manifestations of common variable immunodeficiency (CVID), several researchers have proposed combinations of clinical and immunological biomarkers in order to refine the diagnosis and to provide more personalized follow-up and treatment strategies that may improve the prognosis of the individual patient (1–4). A recently proposed biomarker for CVID diagnosis is the quantification of serum free light chain (sFLC) (5–8).

The key-shaped structure of immunoglobulins (Ig) as originally described by Ehrlich (9), consists of four polypeptide chains, two pairwise identical copies of both heavy (H) and light (L) chains, the latter being named kappa (κ) or lambda (λ) chains (10). This "key" opens up a wide range of processes associated with innate and adaptive immunity. Among these processes stand out the direct neutralization of an almost unlimited number of antigens and toxins, autoantibodies, modulation of fas death receptor, binding to lectins, modulation of the complement cascade, regulation of monocytes/macrophages, activation of NK cells, regulatory T cell expansion, suppression of T and B cell activation, suppression of cytokines, neuroregulatory effects and increased sensitivity of steroids (10, 11). However, functions related to this "master key" are not completely known. L chains are incorporated into Ig molecules during B-cell development. Initially, large pre-B cells express a pre-BCR that is assembled from antibody μ H chains and surrogate L chain (VPREB1 and IGLL1). At the next stage, in small pre-B cells, bona fide L chains (κ and λ) undergo recombination and when this results in a productively recombined L chain, it is expressed together with μHC forming a BCR on the surface of pre B-cells (**Figure 1**) (12). Production in excess of L chains occurs throughout B-cell development till plasma cells, where they bind to H chains, excess L chains enter the bloodstream as FLCs. Secretion of L chains would reflect B cell activation (13, 14).

In healthy individuals, small amounts of both free L κ and λ chains can be found (κ = 3.3–19.4 mg/l, λ = 5.7–26.3 mg/l), with a normal κ/λ ratio ranging between 0.26 and 1.65 depending on the technical assay (5). These ranges were suggested using reference serum samples from 282 healthy donors between the ages of 21 and 90 years (15), based on the polyclonal Freelite assay.

sFLC quantification may indicate the presence of B cell clonality and is widely used in clinical practice for the diagnosis of B-cell lymphoproliferative disorders (B-CLPD), in particular the progression of monoclonal gammopathy of undetermined significance (MGUS) to multiple myeloma (MM), as well as a marker of neuroinflammation, for instance, in multiple sclerosis (16–23). Moreover, dysbalance in sFLC is used as a prognostic marker of various B-CLPD, such as chronic lymphocytic leukemia (CLL), B cell non-Hodgkin lymphomas (NHL), as well as for real-time monitoring of response to treatment and disease progression (5, 17, 24–27). Due to the inherent immunological alterations in a relevant proportion of PID patients, such as polyclonal B cell proliferation, it is particularly challenging to make an early diagnosis of B malignancy in these patients. Interestingly, alterations of sFLC in PID patients (κ/λ ratio), especially in CVID, correlate with clonal processes (6, 7, 13).

Here we sought to validate previous studies on the diagnostic and prognostic value of sFLC. Secondly, we suggest the sum κ + λ as a practical combined biomarker of CVID diagnosis and other potential applications for follow-up and prognosis. Finally, we present a hypothesis on the possible scenarios underlying very low sFLC in CVID and discuss potential experimental and clinical approximations.

MATERIALS AND METHODS

sFLC levels were prospectively evaluated in a cohort of 100 primary immunodeficiency (PID) patients and in 49 patients diagnosed with a hematological malignancy referred to study of secondary immunodeficiency (SID) at the Clinical Immunology Dept., Hospital Clínico San Carlos of Madrid, Spain. All PID patients fulfilled the ESID registry diagnostic criteria (28).

sFLC κ and λ chains were quantified by nephelometry (FREELITE, The Binding Site Group Ltd., Birmingham, United Kingdom), according to the manufacturer's instructions, using a BNII nephelometer (Siemens Healthcare Diagnostics, Camberley, Surrey, United Kingdom).

SPSS statistics software (Chicago, IL, United States) was used for descriptive and statistical data analysis. Pearson's correlation coefficient was used to assess the correlation between variables. $p < 0.05$ was considered statistically significant. Receiver operating characteristic curve (ROC curve) and contingency analysis were performed using GraphPad Prism version 8.3.0 for Windows, GraphPad Software, La Jolla, CA, United States[1].

Approval for the study was obtained from the hospital institutional Ethics Committee for PID and SID projects (19-284-E and 19/219-E), respectively. Written informed consent was obtained from all patients for inclusion in the study protocol.

RESULTS

sFLC Discriminates CVID From Other Primary Immunodeficiencies

We studied sFLC in 100 patients with different PIDs (selective IgA deficiency $n = 38$, unclassified antibody deficiency $n = 27$, CVID $n = 26$, Good syndrome $n = 2$, 22q11.2 deletion syndrome $n = 2$, complement system deficiency $n = 2$, X-linked hypogammaglobulinemia $n = 1$, hyper IgM syndrome $n = 1$, and Kabuki syndrome $n = 1$) as part as routine immunological work-up. CVID patients showed significantly lower κ and/or λ values in comparison to other PIDs (mean: κ: 1.39 ± 1.7 mg/L and λ: 1.97 ± 2.24 mg/L *versus* κ: 13.97 ± 5.88 mg/L and λ: 12.92 ± 7.4 mg/L, respectively, $p < 0.001$ both) (**Figure 2**). The sum of kappa and lambda (sum κ + λ) in CVID patients was 7.25 ± 7.90 mg/L versus 26.44 ± 13.25 mg/L respect to other PIDs ($p < 0.0001$).

[1]www.graphpad.com

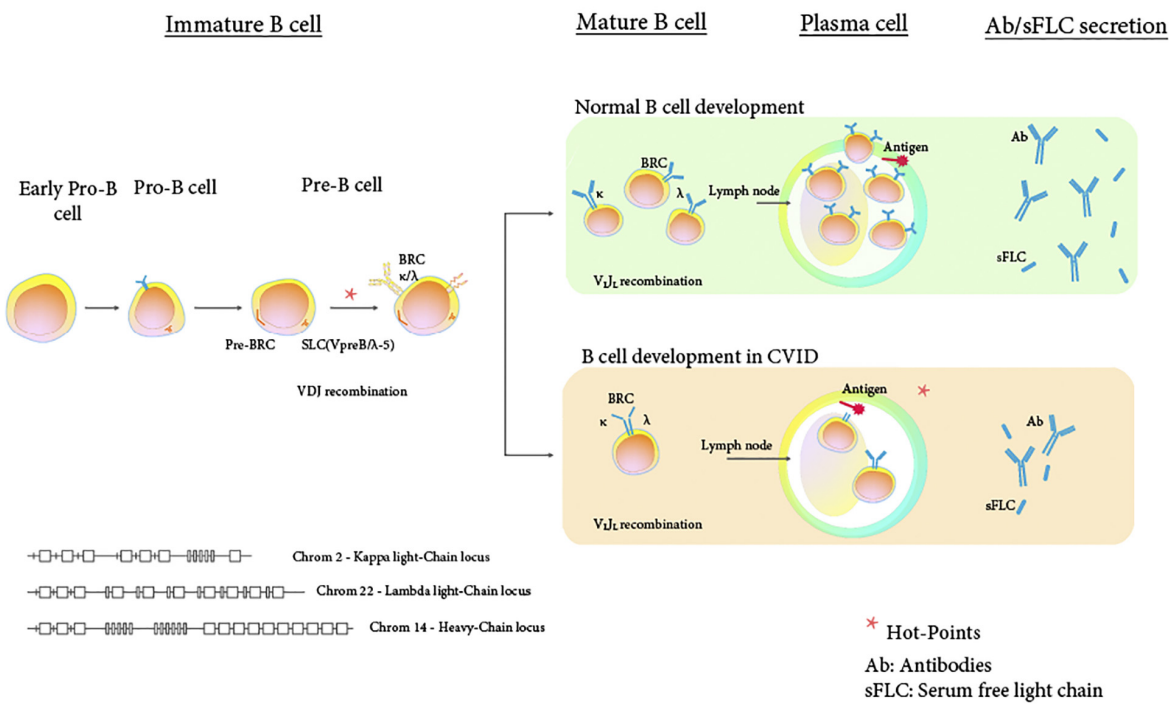

FIGURE 1 | Potential hot-points in the development of sFLC synthesis, assembly and secretion. Scheme of the B cell maturation and differentiation, where an alteration in the rearrangement of the receptors of the pre-B cells, could condition the inadequate production of immunoglobulin light chains and, consequently, the defective expression of immunoglobulins. Modified from Winkler and Mårtensson (12).

When we analyzed the four previously described patterns of sFLCs in CVID (6–8, 29): 53.84% (14/26) disclosed the $\kappa^-\lambda^-$ pattern; 30.76% (8/26) the $\kappa^+\lambda^+$ pattern; 7.69% (2/26) the $\kappa^-\lambda^+$ pattern; and 7.69% (2/26) $\kappa^+\lambda^-$ pattern.

We then tested sFLC values in pure commercial gammaglobulin preparations, detecting mean levels of 4.15 mg/L of κ and 1.59 mg/L of λ, sum κ + λ 5.74 mg/L. However, it was insignificant when gammaglobulin was diluted 1:10, as found in normal plasma.

sFLCs May Aid in the Diagnosis of Secondary Immunodeficiencies

Regarding the comparison of sFLC expression in SID and CVID patients, we evaluated 49 patients with hematological malignancy: CLL (n = 12), NHL (n = 22), MGUS (n = 12) and MM (n = 3). CVID patients showed significantly lower kappa and/or lambda values than SID (mean: κ 1.39 ± 1.7 mg/L versus κ 20.9 ± 22.8 mg/L, $p < 0.001$; and λ 1.97 ± 2.24 mg/L versus λ 12.8 ± 8.7 mg/L, respectively, $p < 0.001$). When comparing the κ/λ ratio in both cohorts, CVID κ/λ ratio was significantly lower than SID (0.94 versus 1.91, $p < 0.005$). The sum κ + λ in CVID was also significantly lower than SID patients (7.25 ± 7.90 mg/L versus 28.25 ± 26.24 mg/L, $p = 0.0002$).

In our SID cohort to B-CLPD, 7 patients with CLL and NHL that showed very low or undetectable sFLC at diagnosis of malignancy were highly suspicious of an underlying CVID based on history of infections since childhood (n = 2) or suspicious family history of PID (n = 2), which were not diagnosed at the time, which could only be confirmed if PID-predisposing gene defect were found (30).

ROC Curves

The diagnostic performance of a CVID-pattern (i.e., diminished levels of free L κ and/or λ in respect to the reference range) was evaluated for the diagnosis of CVID with respect to other PID and SID, showing a sensitivity of 76.00%, specificity of 85.71%, positive predictive value (PPV) 52.78% and negative predictive value (NPV) of 94.4% for CVID diagnosis.

We then compared the diagnostic value of the sum of free L κ + λ levels in this setting. ROC analysis disclosed an area under the curve (AUC) of 0.894 (SD 0.031; 95% CI: 0.83–0.95, $p < 0.0001$), with optimal cut-off of 16.7 mg/L for the sum κ + λ giving the highest combination of sensitivity (92%) and specificity (75.6%), with NPV (97.8%). The Relative Risk (RR) found for CVID was of 20.35-fold (95% CI 5.630–75.93) for patients with sum κ + λ levels below 16.7 mg/L (**Supplementary Figure S3**). The frequency of patients below the recommended sum κ + λ cut-off of 16.7 mg/L was 92% of CVID patients; 10% of other PIDs; and 28% of SID ($p < 0.0001$, both).

Comparison of sFLC Values With Clinical Associations in CVID

We observed a significant correlation between $\kappa^-\lambda^-$ pattern and $CD27^+IgD^-IgM^-$ switched-memory B cells ($p < 0.05$). We did

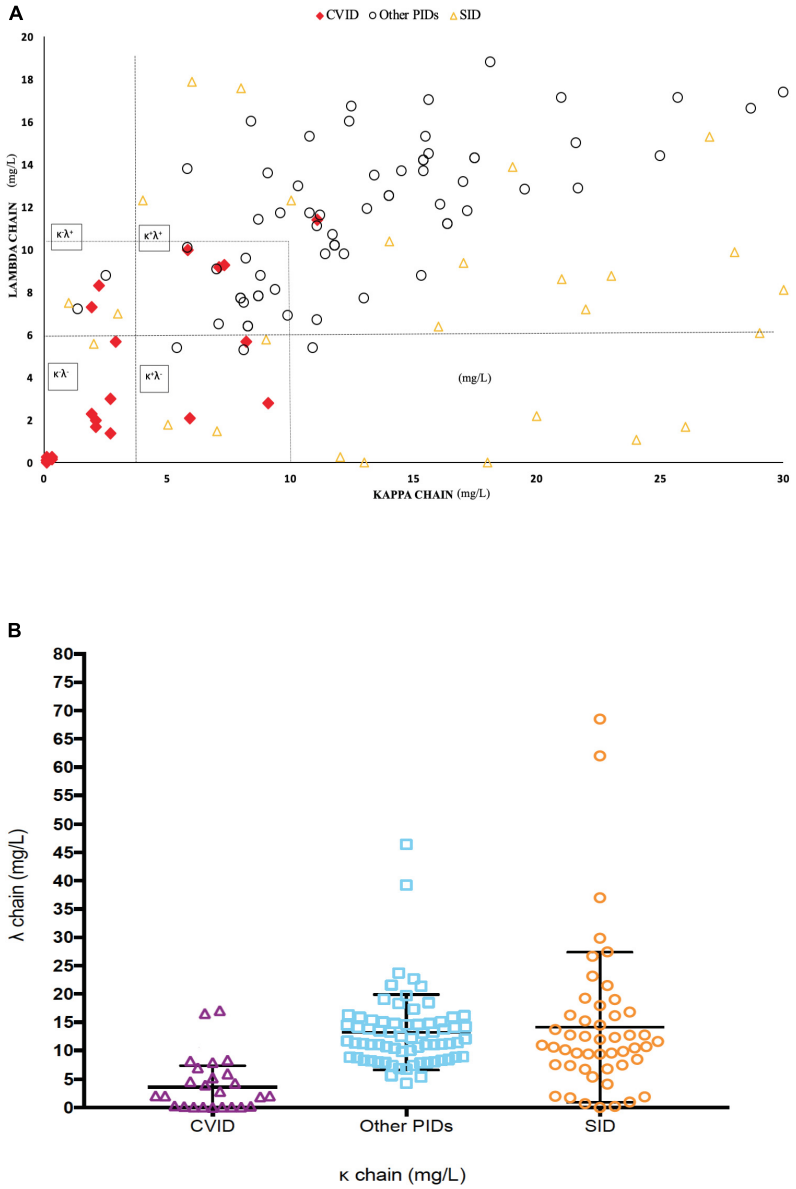

FIGURE 2 | (A) Distribution of lambda and kappa chains among CVID, PID and SID patients (*N* = 100). **(B)** Distribution of sFLC concentration (sum κ + λ) in CVID *versus* other PIDs and SID.

not find statistical significance between specific sFLC patterns and age (p = 0.20), sex (p = 0.30), clinical onset (p = 0.15), CD21^{low} B cells (p = 0.76) or Ig levels at diagnosis of CVID (p = 0.94). We then compared sFLC patterns ($\kappa^-\lambda^+$, $\kappa^+\lambda^-$, $\kappa^-\lambda^-$, $\kappa^+\lambda^+$) with clinical associations in CVID patients. We found that $\kappa^-\lambda^-$ pattern was highly prevalent in CVID patients with enteropathy (66.66%, 4/6 patients, p = 0.01), splenomegaly (50.00%, 7/14 patients, p = 0.2) and bronchiectasis (68.75%, 11/16 patients, p = 0.1), compatible with the association described by other groups (6, 7, 29).

Regarding clinical associations in CVID patients using the sum κ + λ below 16.7 versus above this cut-off, 28.57% (6/21 patients) presented enteropathy versus 0% (p = 0.1), 57.14% (12/21) splenomegaly versus 40% (p = 0.4) and 66.66% (14/21) bronchiectasis versus 40% (p = 0.2).

DISCUSSION

sFLC as a Diagnostic Tool in Primary Immunodeficiencies

Compatible with previous studies, a similar distribution of normal sFLC levels among all PID was observed, except for CVIDs, with significantly lower κ and/or λ values than other PIDs ($p < 0.001$). To better define the most optimal cutoff level for sFLC in our population, we used the sum κ + λ of 16.7 through

standard statistical ROC analyses for discriminating CVID from other PID and SID, AUC of 0.894 ($p < 0.0001$, for both). The sum κ + λ provided a high sensitivity, specificity and NPV for CVID diagnosis.

Several groups have pioneered a greater understanding on the role of sFLCs in the clinical scenario of PIDs (6–8, 29). While many primary antibody deficiency (PAD) share a common profile of hypogammaglobulinemia, it has not been fully elucidated why only CVID presents a characteristic profile of low or undetectable sFLC (6–8, 29). Unsworth et al. described very low sFLCs in 18 out of 20 PID cases, CVID being the commonest diagnosis (16/20), followed by X-linked agammaglobulinemia (XLA, 2/20). Hyper-IgM syndrome (HIGM, 1/20) and non-HIGM with raised polyclonal IgM (1/20) showed normal sFLC values. The common denominator for all 20 patients was antibody immunodeficiency and associated increased frequency of bacterial infections (8). Scarpa et al. have recently described significant variations in sFLCs values in a wide cohort of PID. CVID patients showed decreased or undetectable values of sFLCs compared to normal values in patients with unclassified antibody defects ($p < 0.0001$) (7). Their findings were similar to those described in other studies with different PIDs cohorts (6–8, 29, 31, 32). Regarding XLA, there are contradictory results in the different cohorts described, ranging from low to normal sFLC, which may depend on the genetic defect (33).

In the present study, we used nephelometry with the same test, reagents, and platforms described by Scarpa et al. and Unsworth et al. in PIDs cohorts (7, 8), which reduces the variability among results. The technique used has shown the highest sensitivity for sFLC, with detection up to 0.1 mg/L for both κ and λ as low reference levels (5). However, it could be interesting to design comparative studies between the different techniques currently available for measuring sFLC in the PID setting.

The marked decrease in sFLCs could reflect a profound damage, both quantitatively and functional of the BCR during lymphocyte differentiation in CVID. Moreover, a dysfunctional BCR might be at the origin of a lymphoproliferative or self-reactive status in this patients' population (12). In general, the production of both L chains increases during infections or inflammatory states, with higher absolute concentrations but without change in κ/λ ratio (5, 6, 8). Infection and inflammation are very common and define the clinical phenotypes "cytopenia," "polyclonal lymphocytic infiltration," "unexplained enteropathy," and "no disease-related complications (only infections)" of CVID patients (3). We could infer that CVID patients with chronic inflammatory phenotype would present with high levels in a $\kappa^+\lambda^+$ pattern. Scarpa et al. analyzed the association of sFLCs patterns ($\kappa^-\lambda^+$, $\kappa^+\lambda^-$, $\kappa^-\lambda^-$, $\kappa^+\lambda^+$) and CVID clinical phenotypes. Counterintuitively, infectious and inflammatory phenotypes were more frequently observed in CVID patients with low or absent levels of sFLC ($\kappa^-\lambda^-$) (6, 7, 29). Likewise, we found that enteropathy, splenomegaly and bronchiectasis were more prevalent in the $\kappa^-\lambda^-$ pattern in our cohort, although the non-significance in splenomegaly and bronchiectasis could be due to the small sample size (**Supplementary Figure S1**). Our findings did not demonstrate statistical significance between specific sFLC patterns and age, sex, clinical onset or Ig levels at diagnosis of CVID. In the study of Compagno et al., $\kappa^-\lambda^+$ pattern was the most represented in CVID (21 out of 46 patients, 46%) with higher risk of mortality derived from autoimmune cytopenias, lymphoproliferation and enteropathy (12/21 patients, 57%), followed by $\kappa^-\lambda^-$ pattern (15/46 patients, 33%) with a trend to present splenomegaly (6/15 patients, 40%) and malignancy (5/15 patients, 33%) (6). Scarpa et al. hypothesized that low sFLC levels may be an epiphenomenon of a higher degree of impairment in B cell differentiation, with reduced B cell class-switch affecting immunoglobulins' production (7). Altogether, these findings support that diminished sFLC values observed in CVID are associated with this pathology and can be used as an accessory diagnostic tool to support CVID diagnosis. However, the clinical significance of these patterns is still under study and needs further validation.

Comparison of sFLC Patterns With Other CVID Biomarkers

There is no clear correlation between sFLC with all serum Ig levels at diagnosis in the different PIDs groups studied (7, 8, 29). Scarpa et al. described a direct association of IgA and IgM with serum κ and λ chain concentrations in CVID but not in control groups, while no association between sFLC and serum IgG neither in CVID or control groups (7). Unsworth et al. did not find correlation between IgG and IgA values with sFLC concentrations in their PID cohort (8). Hanitsch et al. described significantly lower IgG levels in $\kappa^-\lambda^-$ CVID, although IgA and IgM levels were not different (29).

There are controversial results regarding sFLC with B cell phenotype in CVID patients. We observed a significant correlation between $\kappa^-\lambda^-$ pattern and class-switch $CD27^+IgD^-IgM^-$ memory B cells ($p < 0.05$), without association between $CD21^{low}$ B cells and sFLC patterns. In contrast, Compagno et al. described a significant decrease in numbers of switched memory, marginal zone, $CD21^{low}$ B cells in the κ-λ- pattern, and a marked decrease of the subsets linked to B-cell activation and Ig production, while no correlation with transitional B cells (6). IN contrast, Scarpa et al. showed the highest frequency of $CD21^{low}$ B cells in κ-λ- group (7). The clinical association derived from these results warrants further study.

Most of our patients were on Ig replacement therapy (IgRT), and serum testing at CVID diagnosis and pre-infusion. Commercial gammaglobulin preparations of pooled normal IgG did contain detectable κ and λ sFLCs, similar to previously published data (8), thus it seems unlikely that they may affect the results, since all patients had normal renal function and the half-life of sFLC in the circulation is 2–6 h (5). IgG infusions are typically repeated every 3–4 weeks so that pre-infusion concentrations measured are likely to only contain sFLC produced by the patient's immune system. Likewise, the multi-time measurement of sFLC in order to determine intra-individual variability showed no difference in our patients (data not shown).

sFLC and the Dilema Between Primary and Secondary Immunodeficiencies

There is a cancer-immune paradox in PID described by some authors (34). The type of malignancy seems to be highly dependent on the specific PID, the age of the patient, and chronic infectious stimuli or dysbiosis involving complex pathogenic mechanisms (35). Cancer is 1.4 to 5-fold higher in registry-based PID studies respect to general population (36, 37), from which 70% corresponds to lymphoid malignancy (38, 39). Individuals with CVID are at 5 to 10-fold higher risk of developing hematological malignancies (36, 37, 40), while unexpectedly lower incidence on most common cancers than general population has been described (41, 42). Cancer is a leading cause of mortality in PID, and thus early diagnosis and treatment of malignancy is a priority (22, 43). When comparing the κ/λ ratio in both cohorts, SID showed significantly higher κ/λ ratio ($p < 0.005$), as expected (7, 29, 43). In CVID patients, the κ/λ ratio is usually normal. In CVID, a B-CLPD can be the first and only clinical manifestation and thus the diagnosis of PID versus SID represents a difficult clinical dilemma. Low κ and/or λ values at hematological malignancy diagnosis might be pointing an underlying CVID. There are no data on the "potential PID patients" in the whole pool of patients with B-CLPD, which may justify to investigate an underlying PID as cancer predisposing factor (35, 40, 41, 44). Also, κ/λ ratio could be important in the follow-up of CVID patients, and hence an altered κ/λ ratio or a sudden increase in sFLC values may be an indicator for further investigation (blood smear, LDH, serum β2-microglobulin, PET-TAC, etc.) that allows appropriate and timely strategies. We consider that the κ/λ ratio behaves like a more reliable marker than the isolated determination of the sFLC when comparing both cohorts.

The Key to CVID: Distinctive Light Chain Defect

The extremely low sFLC in CVID might be explained by different reasons: (i) the lowest the plasma cells numbers, the lowest secretion of sFLC; (ii) increased elimination of sFLC; (iii) altered rearrangement, assembly or secretion of LC during B cell ontogeny; (iv) an intrinsic defect of plasma cells secretion of light chains. To address precisely the first argument, we should quantify plasma cells in the bone marrow, which is not feasible. Indirect measures of total peripheral B cells, class-switched B cells or total serum IgG did not explain sFLC (**Supplementary Figure S2**). We discarded the second argument, since none of the patients had renal or other protein loss. The two last hypothesis imply that the low levels of sFLC in CVID patients may reflect an intrinsic alteration affecting normal production, assembly or secretion of L chains into Ig molecules, which points either to specific defects in plasma cell differentiation (45), or stretches the way back to a critical early event during B cell differentiation. Ig genes are first rearranged in early B cell development through the V(D)J recombination in the liver and then bone marrow and then further modified upon antigenic encounter through the somatic hypermutation (SHM) process in germinal centers of lymphoid nodes. We hypothesize that different underlying mechanisms might correlate with different sFLC patterns, which we discuss below according to clinical and immunological observations and experimental published data:

1. Early B cell defect: Particularly in cases where sFLC are undetectable, L chains should be more profoundly affected than H chains, for which a revision of genetic and molecular processes in the generation of Ig diversity is required. L chains of Ig are encoded in different multigene families from H chains and in different chromosomes: κ in chromosome 2p11.2, λ in chromosome 22q11.2 and H in chromosome 14q32.2, Several potential genetic variations might occur during this process (10, 31), affecting rearrangement of H and L chains in CVID (germline polymorphisms, allelic variants, insertion, deletion, etc.) (14, 46). At pre-B cells stage, first phase (antigen-independent) rearrangement of the H chain occurs, which reacts with light chain-like molecule called surrogate L (SL) chains by allelic exclusion (14, 46, 47). During the cell division cycles, the composition of the pre-B cell receptors (BCR) in the daughter cells engender successful production of a complete κ or λ light chain and further allows the expression of IgM on B cell surface (10).

The pre-BCR is a heterodimer composed of a H chain covalently associated with a surrogate light chains (SL) chain, a temporary common light chain composed by two non-covalently associated proteins, namely lambda-5 (λ5) and V-preB, which together have structural homology with conventional L chains (48). VDJ recombination of the H chain (pre-B cell) precedes pairing with SL chains, proliferation of large pre-B cells and subsequently L chain rearrangement (**Figure 2**). At this stage (at pre-BI to pre-BII or at pre-BII cell to immature B cells for pre-BCR), any transcriptional error in the SL chains, involving the region of the interchain bond during pre-B cells, would affect the correct linkage of SL to H chains and results in lack of LC. Conley reported the first patient with autosomal recessive mutation in the λ5 gene causing severe B cell deficiency and agammaglobulinaemia (33). The mutant λ5 resulted in impaired protein folding and secretion of Ig. It is conceivable that alteration of the players of this highly coordinated process at the pre-BCR stage may result in complete lack of secretion of L chains and undetectable sFLC. Isolated lack of single L chains occurs lately during this sequence. Complete absence of κ chain with normal Ig concentrations has been reported in a patient due to a heterozygous point mutation (1288 GG) that generated an amino acid substitution from Cys to Gly in the protein sequencing, causing an abnormal folding of the polypeptidic constant region of κ chain (31). In this patient, lack of κ chains determined a reduced antibody repertoire despite normal Ig concentrations, associated to recurrent bacterial infections. A correct class switch of H chains requires that the functional CH genes,

located at one end of the rearranged H chain, are activated (10, 47). This synergy of mechanisms might explain in part why, in CVID patients, the decrease in sFLC is frequently associated with the inability to generate $CD27^{+}IgD^{-}IgM^{-}$ switched memory B cells. Additionally, an aberrant recombination of the L chain gene repertoire might favor autoreactive phenomena (49), while altered DNA repair controlling this recombination process would entail an increased pool of aberrant protein involves in B-cell oncogenesis (50, 51).

2. Plasma cell defect: acquired or genetic functional B cell defects have been described after TLR, CD40 and BCR-mediated NFκB signaling pathways that account for altered memory B cell phenotype in CVID patients and low Ig (52, 53). Deep sequencing of IgH locus demonstrated restriction of characteristic patterns of IgHV and IgHJ usage depending on the B cell stimulus (54). Specific defects in the terminal plasma cell differentiation were shown in a subgroup of CVID patients at germinal center responses, with diverse mechanisms converging into the block of final step of plasma cell differentiation (45). In addition, gene lesions in MSH5 has been related to a few CVID patients, with defective S region junctions between LC and HC that would take place after antigen encounter (55). Altogether, these diverse mechanisms result in reduced and modulated class-switched B cells and Ig, which may relate to L chains' restriction and low levels of sFLC secretion (**Figure 1**).

Concluding Remarks and Further Perspectives

Our data validates previous studies emphasizing the relevance of sFLC quantification in the diagnosis and follow-up of CVID patients. sFLC behaves as a promising biomarker in the differential diagnosis of CVID with other PID and SID, and κ/λ ratio as a prognostic biomarker associated with specific clinical phenotypes. A cutoff level $\kappa + \lambda < 16.7$ mg/L supports CVID diagnosis. Moreover, κ/λ ratio alteration or a sudden increase in sFLC values may alert lymphoid malignancy and prompt appropriate and timely diagnostic work-out and therapy, a major concern in this patients' population that impacts the survival. Reference values and cut-off points must be validated for each technique and then compare the different available immunoassays to come up with a reference range for each assay in different populations.

We hypothesize that decreased levels of sFLC in CVID patients may reflect an intrinsic early defect at a critical common step of B cell differentiation in the bone marrow affecting SL or L chain assembly or secretion that would affect memory B cell phenotype. Work is ongoing to check the hypothesis rooting this phenomenon by discarding gene defects during early B cell ontology, or intrinsic alterations in the terminal plasma cell differentiation, by *in vitro* differentiation of plasma cells from CVID patients after stimulation with subsequent determination of sFLC production and secretion. Altogether, we provide new evidence that this biologic phenomenon of low κ and λ provides a common feature of CVID, and leaves entirely open the question of whether would it be necessary to revisit the classification of CVID according to it.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethical committee of the San Carlos Clinical Hospital, Madrid, Spain. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KG-H and SS-R designed the study, integrity and analysis of data, and writing of the manuscript. RP, JO-G, and MF-A contributed to the analysis of data. KG-H contributed to the design of the images and figures. MC contributed to the measurement of the serum-free light chain and the analysis of data. All authors reviewed and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.02004/full#supplementary-material

FIGURE S1 | Bar chart comparing sFLCs patterns ($\kappa^{-}\lambda^{+}$, $\kappa^{+}\lambda^{-}$, $\kappa^{-}\lambda^{-}$, $\kappa^{+}\lambda^{+}$) with infectious and inflammatory phenotypes (Enteropathy, splenomegaly, bronchiectasis and cytopenia's).

FIGURE S2 | Scatter plots comparing Kappa light chain concentration against: **(A)** Serum IgG at diagnosis; **(B)** Class-switched Memory B cells (CD19 + IgD-IgM-CD27 +); **(C)** B cells CD19%.

FIGURE S3 | Receiver operating characteristic (ROC) curves for CVID diagnosis with the sum $\kappa + \lambda$ testing. The area under the curve (AUC) for CVID was 0.894 ($P = 0.001$). The use of sum $\kappa + \lambda$ testing identified 24 of 26 cases of CVID, for a sensitivity of 92% [95% confidence interval (CI), 0.83–0.95 ($p < 0.0001$)].

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Trained Immunity Based-Vaccines as a Prophylactic Strategy in Common Variable Immunodeficiency. A Proof of Concept Study

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Abstract: Background. A major concern in the care of common variable immunodeficiency (CVID) patients is the persistence of subclinical or recurrent respiratory tract infections (RRTI) despite adequate trough IgG levels, which impacts the quality of life (QoL) and morbidity. Therefore, the development of new approaches to prevent and treat infection, especially RRTI, is necessary. Objectives. We conducted a clinical observational study from May, 2016 to December, 2017 in 20 CVID patients; ten of these patients had a history of RRTI and received the polybacterial preparation MV130, a trained immunity-based vaccine (TIbV) to assess its impact on their QoL and prognosis. Methods. Subjects with RRTI received MV130 for 3 months and were followed up to 12 months after initiation of the treatment. The primary endpoint was a reduction in RRTI at the end of the study. We analyzed the pharmacoeconomic impact on the RRTI group before and after immunotherapy by estimating the direct and indirect costs, and assessed CVID-QoL and cytokine profile. Specific antibody responses to the bacteria contained in MV130 were measured. Results. The RRTI-group treated with TIbV MV130 showed a significant decrease in infection rate ($p = 0.006$) throughout the 12 months after initiation of the treatment. A decrease in antibiotic use and unscheduled outpatient visits was observed ($p = 0.005$ and $p = 0.002$, respectively). Significant increases in anti-pneumococcus and anti-MV130 IgA antibodies ($p = 0.039$ both) were detected after 12 months of MV130. Regarding the CVID QoL questionnaire, an overall decrease in the score by more than 50% was observed ($p < 0.05$) which demonstrated that patients experienced an improvement in their QoL. The pharmacoeconomic analysis showed that the real annual direct costs decreased up to 4 times per patient with the prophylactic intervention ($p = 0.005$). Conclusion. The sublingual administration of the TIbV MV130 significantly reduced the rate of respiratory infections, antibiotic use and unscheduled visits, while increasing specific IgA responses in CVID patients. Additionally, the CVID population felt that their QoL was improved, and a decrease in expenses derived from health care was predicted.

Keywords: prophylaxis; TIbV; CVID; quality of life; MV130

1. Introduction

Common variable immunodeficiency (CVID) is one of the most common symptomatic and heterogenous primary immune disorders (PID) [1–4]. The hallmark of the disease is a defect in specific antibody production and hypogammaglobulinemia of at least two isotypes (IgG and IgA/IgM). The pathogenesis of CVID remains unknown for most patients, with 15% of cases pointing to T-B co-stimulation and BCR-dependent gene lesions [2,5–7]. Over 90% of CVID patients suffer from increased susceptibility to infections by different pathogens that affect several systems [1,8,9]. Additionally, the prevalence of bacterial infections might be falsely decreased due to antibiotic prophylaxis in patients with specific conditions [10]. Recurrent respiratory tract infections (RRTI) are very frequent in CVID [1,11]. Recurring infections and subclinical infection are common despite adequate immunoglobulin replacement therapy (IgRT) and can predispose patients to develop chronic lung diseases, like bronchiectasis, interstitial lung disease, granulomatous inflammation, and obstructive/restrictive lung disease, which are a leading cause of both morbidity and mortality in CVID patients [12–16].

Antibiotic prophylaxis in patients with CVID has been shown to improve lung function, reduce the risk of infection-related hospitalization and improve patients' QoL in a recent double-blind placebo-controlled trial [17–19]. However, the risk of bacterial colonization, development of complicated pneumonia, invasive infections or infections by atypical microorganisms was not fully studied [17,18,20]. Antimicrobial resistance (AMR) represents a significant health concern worldwide. It is unclear whether PID patients are more susceptible to developing AMR [17,20,21], even though the use of long-term antibiotic prophylaxis is common in the management of PID patients. Indeed, prolonged administration of antibiotics can result in AMR to Gram positive and Gram negative pathogens, such as Group B streptococcus or *Klebsiella pneumoniae*, respectively [21]. In addition, antibiotics have limitations against several infections, mainly due to viruses, are detrimental to the already altered microbiota in PID, and are not free of adverse effects in the long-term. In this setting, complementary or alternative strategies for preventing infections in this highly susceptible population is a priority.

Immunotherapy with trained-immunity based vaccines (TIbV) are meant to enhance innate cross-protection to heterologous pathogens and to induce more efficient adaptive responses against the specific pathogens contained in the vaccine [22–24]. Their use in PID has not been previously reported. Sublingual administration of fully inactivated bacteria has been proposed as a safe and clinically beneficial strategy of preventing upper and lower respiratory tract infections [23,25,26], although it requires continued, daily administration. MV130 is a TIbV formulated with a combination of whole cell inactivated Gram-positive (90%) and Gram-negative bacteria (10%) that has previously been demonstrated to be a safe and effective immunotherapy against RRTI and induces long-lasting protection [23,25,26]. Bacterial preparations have been shown to elicit systemic and mucosal antigen-specific humoral and cell-mediated immunity [22,27–30]. Sublingual immunization with MV130 has been shown to up-regulate Th1, Th17 and IL-10 antigen-specific, as well as non-specific memory $CD4^+$ T cell responses in vitro and ex vivo [25,26]. Concomitant up-regulation of IL-10 by MV130 may add immunomodulatory potential in the setting of autoimmune or inflammatory imbalance in CVID [25]. Finally, a recent clinical trial in children with recurrent wheezing, a disease mostly triggered by respiratory viruses, demonstrated that MV130 significantly decreased wheezing attacks in children. MV130 induced trained immunity and elicited an antiviral effect in experimental models with vaccinia and flu viruses [31,32].

We used a proof-of-concept study to determine the beneficial effects of a novel adjuvant therapeutic strategy with a TIbV in CVID patients suffering RRTI despite adequate trough IgG levels and antibiotic prophylaxis.

2. Methods

The present study is a single-institution retrospective observational study conducted at the Clinical Immunology Department of the Hospital Clínico San Carlos, Madrid, Spain, from May 2016 to December 2017.

Approval for the study was obtained from the Hospital Clinico San Carlos institutional research Ethics Committee (16/511-E). Written informed consent was obtained from all patients for inclusion in the study protocol.

2.1. Population

Data from a cohort of 20 patients with a definite diagnosis of CVID, aged between 18 to 65 years, and serum IgG above 600 mg/dL on IVIg therapy were recorded. The exclusion criteria were: patients with lymphoproliferative disorders, asthma treated with inhaled or systemic corticosteroids, inability or unwillingness to provide written informed consent or significant medical or psychiatric illness that, in the opinion of the treating clinician, precluded participation.

Subsequently, the cohort was subdivided into two groups according to whether or not the patient presented with RRTI, which was defined by ≥3 episodes of upper (rhinitis, sinusitis, otitis, pharyngitis, tonsillitis) or lower respiratory infection (bronchitis), ≥2 episodes of severe respiratory infection (pneumonia), or the need for antibiotics for almost two months/year in the previous 12 months as described elsewhere [9,10]. This group was referred to as the RRTI-Group. According to our clinical routine protocols at the time, the RRTI-group (n = 10) had received a TIbV MV130 (Bactek®, Inmunotek, Madrid, Spain) daily for 3 months. According to the drug data sheet, the MV130 sublingual immunotherapy was indicated for recurrent infections. MV130 is composed of whole complete inactivated bacteria, as follows: 90% Gram-positive bacteria such as *Staphylococcus* spp., *Streptococcus pneumoniae*, and 10% Gram-negative bacteria such as *Klebsiella pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae*.

As part of our regular medical follow-up, RRTI-group was evaluated before and at 12 months of sublingual prophylaxis, with a complete medical history, physical exam and immunological profile. The sera were stored at −80 ºC. The remaining patients (n = 10) that did not fulfil the RRTI-Group criteria were designated as the Non-RRTI-Group. This group was considered as a control group for the comparison of the immunological CVID profile (Figure 1).

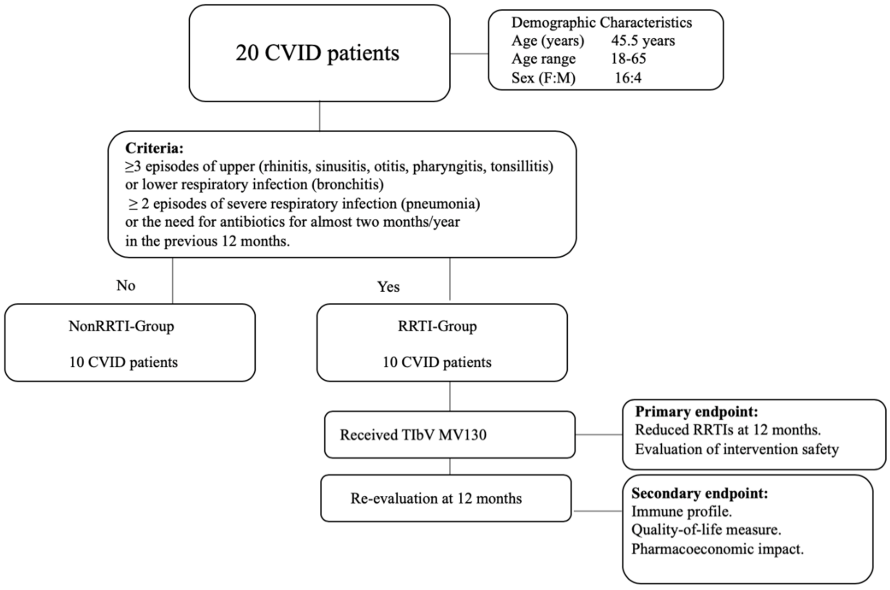

Figure 1. Flow chart of the classification of common variable immunodeficiency (CVID) patients according to clinical criteria.

2.2. Immunological Assessment

We measured the presence of specific serum IgA and IgG against MV130 and *S. pneumoniae* (the main Gram- positive component) in the RRTI group by ELISA. The samples were stored frozen at −80 °C before processing. Briefly, 96-well non-tissue culture-treated plates were pretreated with 100 µL of poly-L-lysine (stock at 0.01%, 1:1000 dilution) (Sigma-Aldrich) for 1 h under UV light and coated with the appropriate whole-cell heat-inactivated bacteria or polybacterial mixture (300 nephelometric turbidity units (NTU), ~10^9 bacteria) overnight at 4 °C, and subsequently incubated with human sera for 2 h at room temperature. IgA and IgG antibodies were detected using the following reagents: biotin rat anti-human IgA or IgG (both from Sigma-Aldrich) and streptavidin horseradish peroxidase (HRP) (Sigma-Aldrich). Peroxidase activity was revealed by the addition of o-phenylenediamine dihydrochloride (Sigma-Aldrich) and the reaction was stopped with HCl 1N. Plates were read on an ELISA reader at 490 nm (Triturus Elisa, Grifols).

Likewise, an extensive panel of Th1/Th2/Th17/Treg cytokine profiles, growth factor, and chemokines was carried out (Pro human cytokine 27-plex kit, Bio-Plex™ from BioRad Inc) at baseline and after treatment (12 months) in the RRTI group and was compared with the Non-RRTI-Group in sera samples, following the manufacturer's instructions. The samples were stored frozen at −80 °C before analysis.

2.3. Endpoints

The primary endpoint of the study was to evaluate the percentage of CVID patients on MV130 who had reduced RRTI in terms of frequency of infections, as well as safety issues. The secondary endpoints were the reduction in the number of antibiotic cycles and in the unscheduled outpatient visits due to infections.

Each CVID subject in the RRTI-group was given the CVID-QoL assessment, before and 12 months after treatment. This was measured by the Health-Related Quality of Life questionnaire for adults with common variable immunodeficiency proposed by Quinti et al. [33]. The questionnaire comprises 32 items with responses given on a 4-point scale, with 0 = "never" and 4 = "always," with higher values generally indicating increasing disability (range, 0 to 160 points).

We evaluated the pharmacoeconomic impact of this prophylactic approach by comparing the characteristics of the RRTI-group before and after vaccination measured by direct and indirect costs. The direct costs estimation was comprised of the medical direct costs and non-medical direct costs. The medical direct costs included those costs attributable to health care visits for the treatment of the disease including primary and secondary care consultations, prescriptions, hospital-based procedures, nature and length of inpatient stay, surgery, and over-the-counter purchases by patients [34]. Non-medical direct costs included travel expenses, home care, etc. The information was collected from the CRF (patient self-reported information) and completed with medical records. These costs were assessed using the official rates most recently published for the public health service for a follow-up period of 12 months. The direct costs were calculated according to the following formula: Direct Costs = Medical direct costs + non-medical direct costs. The evaluation of indirect costs included those derived from work suspension related to the disease. For working patients, an average hourly wage (€14.04) was applied to all absentee time, based on salary cost per productive hour, type of working day, and activity sectors in the National Statistics Institute data [35].

2.4. Statistical Analysis

The integration of all obtained data (from laboratory to clinical data) was done by using bivariate correlations and multivariate analysis in order to detect associations between different variables. Patients were stratified according to the obtained results (different clinical and immunological responses). Normal distribution and testing of outliers were analyzed by means of Shapiro–Wilk and Tukey's range tests, respectively. Data were analyzed by Chi-squared, Fisher's exact, Pearson,

and Spearman correlation coefficient, Mann Whitney U-tests, paired Student's *t* test and Wilcoxon signed-rank test, and one-sample *t* test using SPSS (Chicago, Illinois) and GraphPad Prism software (GraphPad Software, La Jolla, CA, USA). A p-value below 0.05 was considered as statistically significant.

3. Results

3.1. MV130 Significantly Decreased Respiratory Infection Rate, Antibiotic Use and Unscheduled Outpatients' Visits in CVID Patients

All patients suffering from RRTI completed the daily treatment with MV130 for 3 months and were followed up for 12 months. The RRTI-Group treated with TIbV MV130 significantly decreased the median (range min-max) infection rate from 3.00 (1–7) to 0.00 (0–2) ($p = 0.006$) post-treatment. All of the patients had a reduced infection rate in the average follow-up time of 12 months (Figure 2). Thirty percent maintained upper respiratory tract infections. Antibiotic consumption significantly decreased from 5.00 cycles (3–7) to 1.00 (0–1) cycle ($p = 0.005$); the number of infectious-related unscheduled outpatient visits significantly declined from 5.00 (2–6) to 1.00 (0–3) ($p = 0.002$); and work absenteeism decreased from 2.00 (0–3) to 0.00 (0–2) days ($p = 0.005$) in the 12 months after initiation of MV130 administration (Figure 3, Supplementary Materials, Table S1). None of the patients reported any side effects regarding MV130, either local at the site of administration or systemic and no adverse effects were noted during the 12-month observational period.

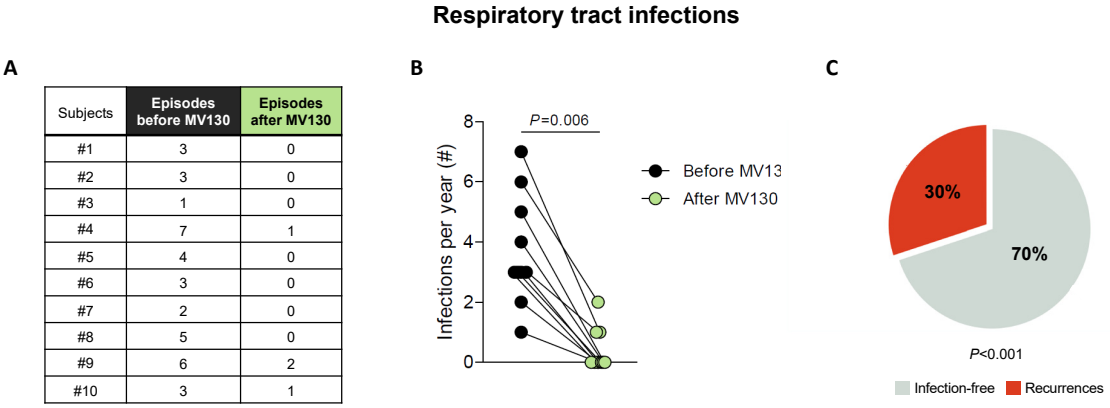

Figure 2. MV130 significantly reduces the incidence of respiratory infections (**A**–**C**). (**A**,**B**) Number of respiratory tract infectious episodes scored 1 year prior to immunization (black) and in the 12 months after the initiation of immunotherapy with MV130 (green). (**C**) Percentage of subjects that remained free of infection (grey) or suffered recurrences (red) in the 12 months following MV130 administration. Data from 10 subjects are shown. (**B**) Lines link paired values. Normal distribution was evaluated using the Shapiro–Wilk test, *p* value was calculated using Wilcoxon signed-rank test. (**C**) *p* value was calculated using Fischer's exact test, compared with rates prior to MV130 initiation (100% of subjects suffering infections).

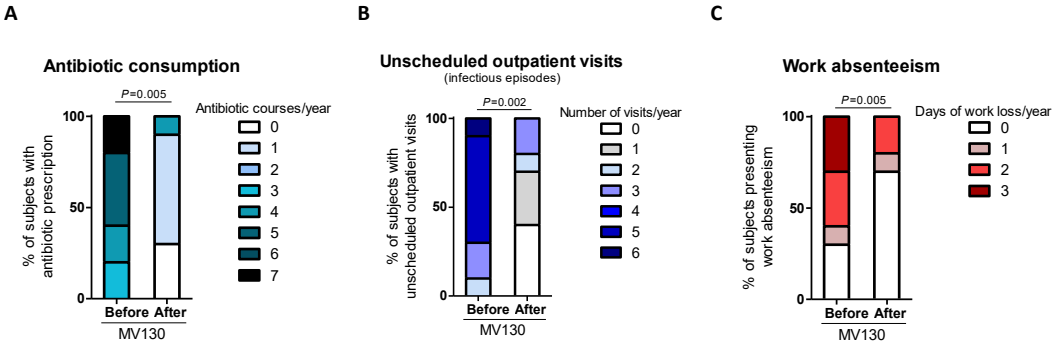

Figure 3. Prophylaxis with MV130 significantly decreases the rate of healthcare resources consumption and work absenteeism. (**A**–**C**) Antibiotic consumption (**A**), visits to emergency unit (**B**) and work absenteeism (**C**) during the year before and after the initiation of MV130 treatment. Bars show the relative number of antibiotic courses (**A**), emergency unit visits (**B**) or days of work lost (**C**) in the total of subjects recorded. Data from 10 subjects are shown. Normal distribution was evaluated using the Shapiro–Wilk test. *p* values were calculated using Wilcoxon signed-rank test.

3.2. Immune Profile

As a preliminary approach, to assess whether sublingual immunotherapy with MV130 triggers a systemic humoral response as well, blood samples were assayed for specific IgG and IgA at baseline and after 12-months of initiating MV130. A significant increase in both anti-*S. pneumoniae* and anti-MV130 IgA ($p = 0.039$) but not IgG ($p = 0.094$) serum antibodies following MV130 immunization were observed (Figure 4). As *S. pneumoniae* represents the main Gram-positive component in MV130, these results point to the induction of a humoral specific response upon bacterial immunotherapy.

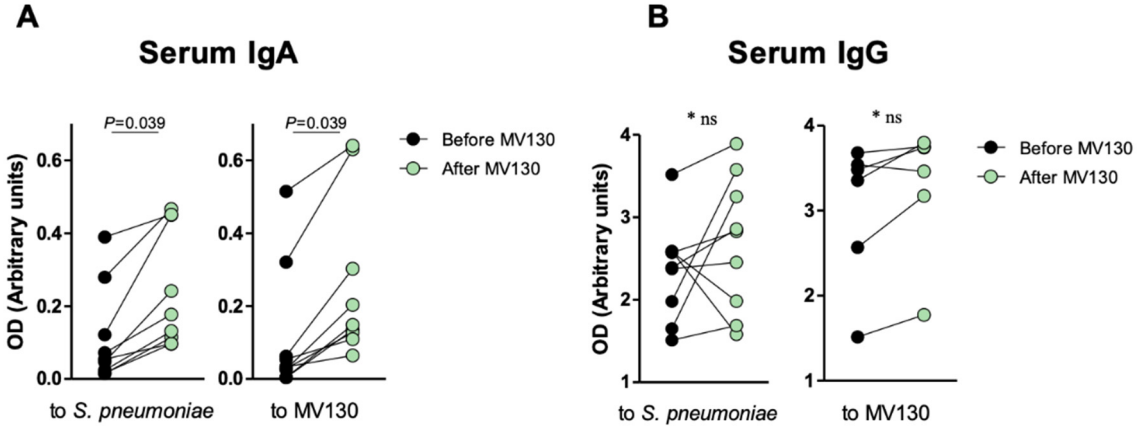

Figure 4. Specific anti-MV130 IgA and IgG antibodies. Prophylaxis with MV130 increases serum IgA antibody production. (**A**,**B**) Serum IgA (**A**) and IgG (**B**) antibodies against *S. pneumoniae* (left panels) or the bacterial mixture (right panels), collected from subjects before and after 12 months following MV130 immunotherapy analyzed by ELISA. Data from 6–10 individuals are shown. Lines show paired values. Normal distribution was evaluated using the Shapiro–Wilk test. *p* values were calculated using Paired Student's *t*-test or Wilcoxon signed-rank test.

We then analyzed the baseline expression of an extensive panel of serum cytokines, chemokines and growth factors in both cohorts (RRTI-group and Non-RRTI group). The RRTI-group showed higher baseline expression of IL-4, IL-17, IFN-γ, MCP-1 (MCAF) and TNF-α, with respect to Non-RRTI group, without statistical differences. Interestingly, when comparing the RRTI-group at baseline with respect to 12 months after MV130, a significantly lower concentration of serum IL-17 (49.49; 0–90.04 vs. 26.85; 0–70.27, $p < 0.05$) with a significant fold reduction in serum IL-17 (0.649, $p < 0.05$); and IL-4 (23.01; 7.03–27.44 vs. 16.92; 5.13–26.35, $p < 0.05$), and fold reduction of IL-4 (0.780, $p < 0.05$) were observed.

This decrease in serum IL-17 restored cytokine levels to those found in non-RRTI individuals (data not shown). The cytokine expression measured pre- and post-immunization in the RRTI-group can be seen in Figure 5.

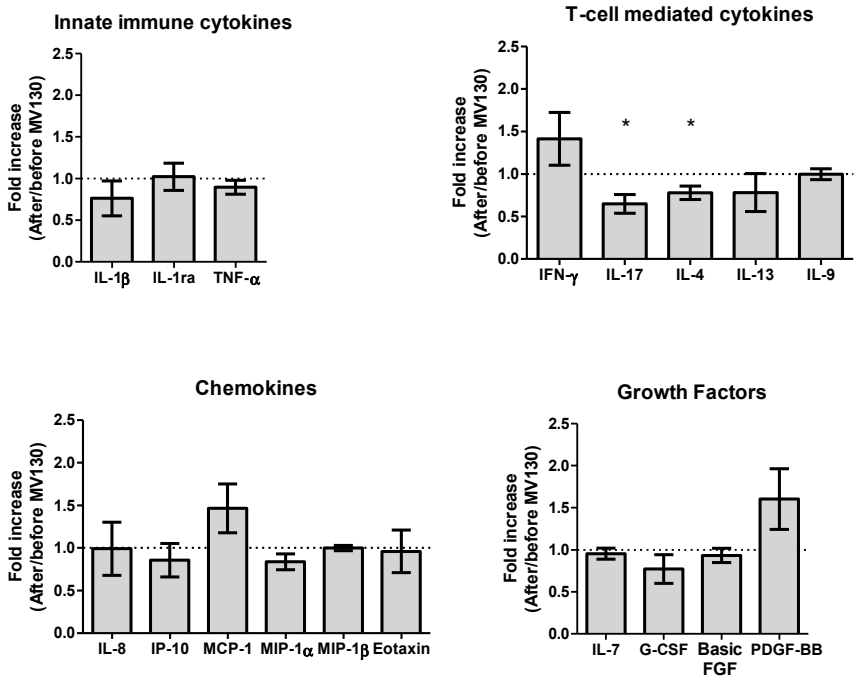

Figure 5. Prophylaxis with MV130 modulates serum cytokine, chemokine and growth factor secretion pattern. Cytokines (upper panels), chemokines (left bottom panel) and growth factors (right bottom panel) secreted in serum one year following MV130 administration determined by Luminex technique. Fold induction relative to basal level (before MV130 treatment) is shown. Undetectable values were found for IL-2, IL-5, IL-10, IL-12(p70), IL-15, GM-CSF, RANTES and VEGF. Data from 9–10 subjects are shown as mean ± SEM of fold increase. Outliers were identified by means of Tukey's range test on represented values. Normal distribution was evaluated using the Shapiro–Wilk test. p values were calculated using one-sample t-test with a theoretical value of 1 (no fold induction). * $p < 0.05$.

3.3. Perceived QoL Improved in CVID Patients after MV130

We evaluated the self-reported quality of life of each RRTI-group patient at baseline and 12 months after receiving prophylaxis with MV130 based on the adapted CVID-QoL questionnaire proposed by Quinti et al. [33]. An overall significant decrease in score was observed (the median (range) of the global score decreased from 47.0 (27–86) to 39.5 (13–86), $p < 0.05$), reflecting an improvement in QoL for patients (Supplementary Materials, Figure S1). The median percentage of pre to post MV130 CVID-QoL improvement was 16.87% (range, 0% to 51.85%). Only one item, "Run out of medications" showed an increase in perception post-treatment while the item that decreased the most was "Difficulty in usual activities".

3.4. Pharmacoeconomic Impact

The economic impact of this prophylactic approach (pre/post-MV130 immunization) was evaluated in terms of direct costs, which were comprised of medical and non-medical direct costs. The indirect costs evaluation included those derived from work suspension related to the disease (Supplementary Materials, Table S2).

Regarding the direct costs, the total average cost for ambulatory care for patients with RRTI is 1656 €/patient, according to the official report of the Spanish Ministry of Health, Consumer Affairs and Social Welfare in 2017 [36]. This amount can increase to 3962 €/patient when hospital admission

is required. The estimated real median annual direct cost is approximately 18,600 €/patient in the RRTI group (Table 1). Taking into account the cost of the intervention, our results showed that the real annual median direct cost decreases up to 4-fold per patient with the prophylaxis intervention of TIbV MV130 (from €18,600 to €4500, $p < 0.005$).

Table 1. Costs of the most frequent conditions affecting CVID patients with RRTI—comparison of the year before and the year after the prophylaxis with TIbV (Euros). (#: number)

Condition	Average # of Episodes before TIbV MV130	Average # of Episodes after TIbV MV130	Cost per Patient per Episode/ Day €	Annual Cost per Patient before TIbV MV130 €	Annual Cost per Patient after TIbV MV130 €	Annual Savings per Patient with TIbV MV130 €
# of RRTIs	3.7	0.4	1656	6127	662	5464
# of physician/hospital/ ER visits	4.4	1.1	1288	5667	1416	4250
# Days Hospitalizations for RRTIs	7	3	792	5546	2377	3169
Cycles of antibiotics	4.8	1	259	1243	259	984
School/work days missed (Absenteeism)	1.6	0.5	14	22.4	7	15
Total per patient				18,606	4722	13,884
Annual cost TibV MV130 prophylaxis					190	13,694

With regard to indirect costs, 14 euros per hour was defined as the cost of absenteeism due to illness, as well as presenteeism due to the reduction in the percentage of productivity [36]. A median of 2 days of absenteeism (the daily workday is 8 h), equivalent to €28, added up to €308 for a cumulative 22 h of labor absenteeism, giving a total of 336 €/patient of annual indirect costs in the pre-immunization RRTI group. These costs are only imperceptibly different (median work absenteeism post-immunization was 0 days) to those calculated after the prophylaxis intervention of MV130 ($p < 0.005$).

4. Discussion

Subclinical and recurrent infections remain a considerable healthcare concern in a subgroup of CVID patients, and they affect the risk of complications and hence the prognosis [10,37]. Bronchiectasis is a consequence of recurrent lower respiratory tract infections and the most common chronic pulmonary complication at CVID patients. It is also the most important cause of morbidity in these patients [11,38,39]. The lung damage could contribute to the high recurrence of infections in a specific group of CVID patients. To our knowledge, this is the first study that shows the beneficial effects of an adjuvant TIbV on the control of recurrent upper respiratory tract infections in CVID patients.

IgRT has been demonstrated to be the best intervention to change the course of CVID by preventing infections [40]. Still, despite adequate serum trough IgG levels, some CVID patients on IVIg/SCIg replacement suffer from subclinical infections and RRTI that require constant or frequent antibiotics and/or they may develop bronchiectasis, which negatively impacts their vital prognosis [40–42]. Therefore, new adjuvant prophylactic approaches to infection, especially in this CVID group are greatly needed.

Most pathogens affecting CVID patients enter the body by the oropharyngeal mucosa. In this proof-of concept observational study, MV130 significantly decreased the rate of respiratory tract infections, the frequency of antibiotic consumption and the frequency of unscheduled outpatient visits. Moreover, specific IgA responses were observed despite the intrinsic antibody defect in CVID. IgA secretion has been strongly associated with decreased rates of respiratory infections, and thus overall mortality risk [43,44]. Significantly higher specific anti-pneumococcal and anti-MV130 IgA antibodies is highly relevant in the context of CVID, especially because these patients are usually low

antibody producers. Specific, significant differences in serum IgA may be explained by the mucosal route of administration of the treatment. MV130 has been shown to increase mucosal IgA secretion [45]. IgA synthesis occurs in mucosal-associated lymphoid follicles through B cell hypermutation and class switch recombination, and is secreted by plasma cells. Secretory IgA (sIgA) is exclusively present on mucosal surfaces and consists of dimeric IgA linked via the J-chain to the secretory components [46,47]. Circulating sIgA constitutes a natural key systemic anti-inflammatory factor due to its ability to interact with DCs through receptors such as ICAM-3, DC-SIGN, favoring the generation of Ag-specific regulatory $CD4^+$ T cells [46,48], which further suggests that IgA has a homeostatic role between commensal microorganisms and pathogens.

Even though the main strength of MV130 as a TIbV may rely on the induction of broad-spectrum, non-specific protection, the data support the hypothesis that specific adaptive responses are also generated that may also correlate with the clinical benefits that result from MV130 administration.

The MV130 polybacterial mixture is prepared by using whole complete inactivated bacteria (90% Gram positive and 10% Gram negative bacteria). This formula was initially standardized according to the ad hoc prevalence of isolated pathogens for the preparation of individualized vaccines for patients with recurrent respiratory tract infections. The Gram positive and Gram negative bacteria act in a synergistic and complementary way in the activation of innate immunity [25]. MV130 has been shown to activate trained immunity in vitro and ex vivo [26,32] by inducing epigenetic and metabolic changes. Regarding the route of administration, mucosal routes for vaccines against respiratory pathogens have been shown to be effective and safe compared to other routes [26,47–51], and they induce a greater immune response in the respiratory tract mucosa.

MV130 may enhance innate immunity at the entry portal of the pathogen. Several studies have highlighted the important role of mucosal preparations formulated with inactivated bacteria in controlling respiratory exacerbations in the setting of chronic obstructive pulmonary disease [52] and preliminary results in patients with RRTI without a defined PID [31,51]. This strategy might provide a potential solution to clinical problems not fully covered by other treatments.

Trained immunity is based on biological processes derived from innate immune memory associated with intracellular signals driving deep metabolic changes and epigenetic modifications that result in reprogramming in cells of innate immunity [53,54]. These effects occur synergistically where various metabolites and metabolic enzymes act as cofactors in the process of epigenetic modification such as transcriptional changes, activation/inhibition of histones, or modulation of cytokine expression [55]. Several therapeutic strategies have been designed to modulate trained immunity [23–26,56]. TIbVs have the potential to improve immune responses by protecting against secondary related or bystander infections, and reversing immunotolerance states as well as chronic inflammatory conditions [22,24,27,29,51].

The main goal of this proof-of-concept study is to generate preliminary data on the beneficial effects and potential risks of these polybacterial mucosal vaccines in order to support the design a larger randomized double-blind clinical trial. The present study has provided an initial evaluation of the safety of the intervention in the target population, which is a major clinical endpoint. The main weakness of this study is the observational retrospective design, which may result in missing variables not recorded in routine clinical practice. However, due to the promising results obtained, this study could help in the design of further studies.

MV130 induces the activation of TLR and NLR signaling pathways in human dendritic cells (DCs), which secrete cytokines such as TNF-α, IL-6 and IL-1β related to trained immunity. These DCs also possess the capacity to promote Th1 and Th17 responses through the RIPK2 and MyD88 signaling pathway under the control of IL-10. In addition, DCs have a large number of pattern recognition receptors that facilitate the synergistic action of the innate and adaptive immune systems [25].

Several studies have identified abnormalities in cytokine secretion in CVID patients, including decreased production of IFN-γ, IL-2, IL-5, IL-7, IL 4, IL-10 or IL-12 [57–62]. IL-10 is essential to avoid excessive deleterious responses, to enhance pathogen clearance, and to keep tissue homeostasis [6,63–66].

No significant changes in serum IL-1β or IL-10 with MV130 were observed. However, we consider that this may be because the cytokine measurement was performed 12-months after MV130 administration, and thus, is reflects the long-term regulation of the proinflammatory status due to the decrease in recurrent infections in this group of patients. The high baseline expression of IL-17 observed in our cohort has already been described in previous studies [58]. However, their clinical significance and the correlation with other biomarkers such as IFN-γ tend to be heterogeneous. Nevertheless, the correct way to observe the real behavior and significance of these results is to carry out in vitro activation of peripheral blood cells of our patients that is stimulated by various ligands, and measure the different expression of cytokines during and at the end of the treatment.

QoL is a multidimensional concept that encompasses several elements such as the physical, psychological and social well-being of individuals. The main point of QoL is to assess an individual's perception of the impact of illness on his/her life at a similar level to that of the clinical factors in the disease's prognosis [67]. RRTI has a critical effect on the QoL of CVID patients. The prophylaxis intervention of TIbV MV130 improved the global perception of QoL in more than 50% of the CVID patients ($p < 0.005$). "Difficulty in usual activities" was the component with the highest post treatment decrease in score. The only element that scored higher after the prophylactic intervention was "Run out of medications". These findings further highlight the positive impact of the prophylaxis with a TIbV such as MV130 on the QoL of CVID patients.

CVID treatment without complications requires IgRT, which implies an annual cost of about 15,700 €/patient. In selected patients, treatment costs may be controlled by modifying the dosage of IgRT, changing the administration route to SCIg or changing the intervals between administrations [68]. The reduced frequency of infections among CVID patients after treatment with IVIg/SCIg translates into health care cost savings [69]. However, a subgroup of CVID patients is unable to improve their infection profile in spite of IVIg/SCIg treatment, consequently, the implementation of an effective alternative treatment can have a direct impact on the abovementioned health care costs of such patients.

Despite the limitations inherent to this observational small study, we show that the clinical benefits of intervention by TIbV are accompanied by healthcare cost savings of approximately 9000–14,000 €/patient per year in terms of hospital, healthcare and lost wages. Considering the high cumulative cost of the treatment of this disease, bacterial immune-stimulation could be an effective strategy to control subclinical infection and respiratory tract exacerbations, and to reduce costs in CVID patients.

5. Conclusions

There is a need to develop alternative or adjuvant strategies to prevent infections in selected CVID patients with subclinical and/or recurrent infections. The preliminary data in our small cohort of CVID patients with recurrent infections despite adequate trough IgG levels show that mucosal vaccines are a potentially useful strategy for preventing infections at the actual point of entry for most pathogens. The study shows enough promise to be repeated in a double-blind clinical trial. The prophylaxis with sublingual TIbV MV130 based on whole cell inactivated bacteria resulted in significant clinical benefits in terms of recurrence and severity of respiratory tract infections, with a concomitant improvement in QoL perception as well as a decrease in health care expenses.

Supplementary Materials: The following are available online at http://www.mdpi.com/2227-9059/8/7/203/s1, Table S1. MV130 significantly reduces consumption of healthcare resources and work absenteeism. Antibiotic consumption (left columns), visits to emergency unit (middle columns) and work absenteeism (right columns) during the year before and after the initiation of MV130. Absolute numbers from 10 subjects are shown. Figure S1. Analysis of the CVID-QoL, before and after treatment with the prophylaxis intervention of TIbV MV130, using the CVID_QoL questionnaire proposed by Quinti et al. [33]. Table S2. Cost assessed, source of resources, source of costs per "unit" and cost calculation used for direct and indirect costs associated with recurrent respiratory infections.

Author Contributions: K.G.-H. and S.S.-R. designed the study, analyzed the data and wrote the manuscript. M.F.-A., J.O.-G. and R.P.d.D. contributed to the design of the methodology. P.S.-L. contributed to the writing of the manuscript and statistical analysis of data and figures. C.M.D.-R. contributed to the measurement of specific antibodies and writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

CVID	Common variable immunodeficiency.
RRTI	Recurrent respiratory tract infections, defined by ≥3 episodes of upper (rhinitis, sinusitis, otitis, pharyngitis, tonsillitis) or lower respiratory infection (bronchitis), ≥2 episode of severe respiratory infection (pneumonia), or the need for antibiotics for almost two months/year in the previous 12 months [9,10].
IVIg	Intravenous immunoglobulin.
SCIg	Subcutaneous immunoglobulin.
QoL	Quality of life.
IP-10	Interferon gamma-induced protein 10.
VEGF	Vascular endothelial growth factor.
TIbV	Trained immunity-based vaccines.
DCs	Dendritic cells

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Proposal of a Combined Prognostic Score for Common Variable Immunodeficiency: Variable Immunodeficiency Score Upfront Analytical Link (VISUAL)

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ABSTRACT

The broad and heterogeneous clinical spectrum that characterizes common variable immunodeficiency (CVID) is associated with quite different disease course and prognosis, highlighting the need to develop tools that predict complications. We integrated the clinical CVID scores by Chapel, Cunningham-Rundles and Ameratunga severity score, in a multivariable VISUAL score (Variable Immunodeficiency Score Upfront Analytical Link) aimed to predict prognosis using individual CVID patient data at baseline. VISUAL score at diagnosis showed adequate performance for predicting infectious and non-infectious severe complications (cluster B) in a cohort of 50 CVID patients from two different centers in Portugal and Spain. Compared to switched memory B lymphocyte phenotype, VISUAL provided a more accurate identification of clinically meaningful outcome, with significantly higher sensitivity (85% *vs* 55%, p=0.01), and negative predictive value (77% *vs* 58%) and AUC of the ROC curves (0.72 versus 0.64), with optimal cut-off level of 10. For every increase of one point in the VISUAL scale, the odds of being in the higher risk category (cluster B) increased in 1.3 (p=0.007). At diagnosis of CVID, VISUAL scores ≥10 showed 8.94-fold higher ODDS of severe prognosis than below this threshold. Time to progression to cluster B of severity was assessed by Kaplan-Meier estimates for the VISUAL score. CVID patients with VISUAL≥ 10 points progressed to cluster B faster than those with VISUAL <10 (*P* = .0002). This prognostic laboratory score may allow close monitoring and more aggressive treatment in patients with scores ≥ 10 on a personalized basis approach.

Key-words: clinical score, common variable immunodeficiency, prognosis, switched-memory B lymphocytes

INTRODUCTION

Common variable immunodeficiency (CVID) is the most commonly symptomatic primary immunodeficiency disorders (PIDD) [1], with a prevalence of 1 in 25,000 to 50,000 individuals. Both genders are equally affected [2,3]. The broad and heterogeneous clinical spectrum that caracterizes CVID is associated with quite different disease course and prognosis, highlighting the need of prediction tools for complications. Combination of biomarkers in scores may assist in providing a more accurate picture of the disease burden in the individual patient. Although these tools are essentially used for research purposes, very few are used in the clinical routine for CVID patient populations. The EUROclass trial was designed to define CVID subgroups according to memory B lymphocyte phenotype, concealing the previous classification schemes of Paris and Freiburg [4]. This classification resulted predictive for autoimmune disorders, lymphoproliferative or granulomatous complications, but did not encompass the extent of manifestations associated with the severity of disease. World CVID experts have proposed that CVID diagnosis should be based on clinical manifestations and laboratory criteria, and then complemented by new biomarkers such as B and T lymphocyte phenotype, lymphoproliferative studies, activation markers and genetic tests for a more precise diagnosis [5–9]. The clinical phenotyping classification proposed by Chapel [7], the scoring system to initiate IgRT proposed by Cunningham-Rundles [10], and the clinical severity score proposed by Ameratunga [11] are widely used in clinical practice.

Here we sought to validate a combined CVID prognostic score (Variable Immunodeficiency Score by Upfront Analytical Link or VISUAL) based on the previous classification systems as an accurate and easy tool to predict prognosis in adults with CVID.

METHODS

Subjects

We conducted an observational study to assess the clinical performance of a prognostic immunological score (VISUAL) retrospectively applied on 50 CVID patients followed at the Departments of Clinical Immunology of the Centro Hospitalar do Porto, Portugal and the Hospital Clínico San Carlos, Madrid, Spain. The study was approved by the Ethics committee of both centers.

Data on initial serum immune globulins and antibody responses to immunization against polysaccharide and protein antigens were collected. For serum immune globulins, we used the age-adjusted normal values [12,13], the values decreased below two standard deviations (<2SD) as criteria for CVID diagnosis and undetectable values (IgM<0.04 g/L and IgA<0.07 g/L, respectively) by routine techniques in our laboratories. Regarding antibodies' responses to immunization: for protein vaccines, we defined adequate response as a 4-fold increase in IgG anti-tetanus toxoid antigen above the pre-vaccination concentration or above 0.15 IU/ml [14–17]; for IgG pneumococcal polysaccharide vaccine responses, a 3-fold increase in anti-PPV titers above the pre-vaccination concentration or above 11 mg/dL, as previously published [18–22]. Data of switched memory (smB) B-lymphocytes ($CD19^+CD27^+IgD^-IgM^-$) and $CD4^+$ T-lymphocytes' proportions and counts determined by multiparametric flow-cytometry as per routine analysis were collected. Normal smB lymphocytes reference ranges were defined between 6%-29% [23–26]. To classify categories of smB lymphocytes we used the cut-off points between categories of 8% of smB according to Paris classification [27], 2% of smB according to the EUROclass study [4], and of 1% using standard ROC analysis in a single-center study [28]. $CD4^+$ T-lymphocytes varies widely in CVID patients with distinctive complications [29]. $CD4^+$ T-lymphocytes counts were categorized according to established HIV immunological stages (<200, stage 1; 200-499, stage 2; ≥500, stage 3) [30]. We further divided stage 3 based on the lower and upper cut-off points considered within normality into low (500-699) and high (>700/Ul) ranges (Table 1).

Clinical information was compiled on each CVID subject, including history of infections, pneumonias; chronic lung disease, bronchiectasis, pulmonary granulomata or lymphoid interstitial pneumonia; severe infections; autoimmune diseases, granulomatous infiltration; presence of splenomegaly; splenectomy, skin and musculoskeletal

manifestations, endocrine, cardiac, kidney and liver disorders, as well as allergies, and history of malignancy, among others [11].

Statistical analysis:

The statistical analyses were performed with the R software (3.6.2) [31] and the packages OptimalCutpoints [32], boot [33] and pROC [34]. The diagnostic performance of the VISUAL score was tested against the Ameratunga's severity score, which was taken as the gold standard. According to the distribution of the Ameratunga's severity score in our series, we decided to categorize this variable into two clusters (A and B). The optimal cut-off of the VISUAL score and the sensitivity, specificity and area under the Receiver Operating Characteristic curve (ROC) of the model were calculated with the ROC method implemented in the OptimalCutpoints package[34]. Kaplan-Meier estimates of the cumulative probability of clinical progression to cluster B were calculated for two groups, established according to the VISUAL cut-off, and were compared with the log rank test (Mantel-Cox). In addition, the VISUAL score was compared with the class-switched B lymphocytes using the McNemar test for the sensitivity and specificity, and a bootstrap method for the AUC. At present, the class-switched B lymphocytes are the best prognostic marker of common variable immunodeficiency. A p value of 0.05 was considered as statistically significant.

RESULTS

Validation of established scores for CVID:

The cohort comprised 50 patients, with gender distribution of F:M 2:1. The median age at diagnosis was 32 years (range, 3-70), and the median of evolution of the disease was 15.9 years at the present time.

Based on the revised CVID classification of clinical phenotyping proposed by Chapel et al. [7], 76% of our patients entered one of the four categories without overlap (cytopenia, 40%; polyclonal lymphocytic infiltration, 19%; unexplained enteropathy, 12%; and no disease-related complications, 5%). The remaining 24% of our cohort showed overlap of 2 clinical phenotypes (mainly cytopenia and polyclonal lymphocytic infiltration, representing 10%). Hence, our data further validate this clinimetric instrument in populations of different ethnic and geographical substrate, similarly to others groups [5,8,35].

We then applied the Cunningham-Rundles' scoring system to guide decisions on IgRT on an individual basis [10]. In our CVID cohort, the median laboratory score was 15 and the median clinical score was 20, resulting in a median cumulative score of 35. Forty-three CVID patients (86%) had laboratory scores of 10 or greater and all had cumulative scores above 16. In 7 patients with laboratory scores below 10 (median of 7), 3 had cumulative scores above 16 and the remaining 4 (8%) between 10 and 16. These results were similar to the those described in the original cohort [10]. All patients on IgRT in our cohort fulfilled IgRT-score criteria, further validating the usefulness of this score in the decision to treat with IgRT.

Proposal and study validation of the VISUAL tool

The VISUAL score was developed as follows: i) a list of candidate variables aimed to predict the clinical severity of patients with CVID was analyzed (serum immunoglobulins, IgG subclasses, production of specific antibodies, memory B lymphocyte phenotype and lymphocyte subpopulations including $CD4^+$ and $CD8^+$ T-lymphocytes, B lymphocytes and NK, C3 and C4 complement factors); ii) only those proved to be statistically significant with P values <0.05 in the multivariable tests (ANOVA) for severity score were included in VISUAL (smB lymphocytes, IgA, specific Ab responses, $CD4^+$ T-lymphocytes); iii) the different components of the VISUAL score

were scored from 1 point for the normal range to 4 points for the absence of each of them (score from 5 to 16); iv) the final VISUAL was calculated as the sum of the individual scores. The suggested VISUAL score for analytical biomarkers is shown in the **Table 1**.

Considering the expression of smB lymphocytes, we observed that 24% of our patients presented normal levels (>10%), 36% of our cohort smB lymphocytes between 8% and 2%; 14% between 2% and 1%, and 26% levels below 1%. Fifty percent of our patients showed undetectable IgA levels, 24% had values < 2SD and 26% presented low to normal levels. Regarding to antibody responses to immunization, 58% of our patients showed inadequate specific antibodies' (Ab) responses to both polysaccharide and protein antigens; 28% had only inadequate polysaccharide Ab responses, while 14% only inadequate protein Ab responses. Forty-four percent of our CVID patients presented $CD4^+$ T-lymphocytes within normal range; 12% had $CD4^+$ values between 500 and 700 μ/mL, 32% of the cohort between 200 to 500 μ/mL and 12% showed levels below 200 μ/mL.

When we applied VISUAL to our cohort, a bimodal behavior was observed, with a median of 10 points **(Supp. Fig.1)**. For clinical validation of VISUAL, we applied the Ameratunga's disease severity score and then confronted both scores. In our CVID cohort, the Ameratunga's severity score disclosed a median of 14 points (range, 8 to 21) and a bimodal behavior [11]. Patients with severe complications or more than one moderate complication amounted to 14 or more score points. The cohort was divided according to the clustering algorithm using 14 points as the severity score **(Supp. Table 1)**, based on median score of 14 and the behavior of the population with severe manifestations, into Cluster A (no severe complications) and Cluster B (severe complications). In our cohort, fifteen patients (30%) have presented at least 1 severe complication, mainly related to lung damage due to bronchiectasis and serious infections, such as meningitis or septicemia. Thirty-three patients (66%) presented moderate complications, mainly complicated pneumonia and related altered gut/nutrition. The remaining 2 patients (4%) presented exclusively mild complications, such as otitis externa or media, or acute sinusitis.

We then compared model fit by cross-tabulation of VISUAL with the frequency distribution of the severity score using Fisher exact test and scatter plots. Correlation

analysis was performed to compare performance between scores and analyzed by K-Means clustering algorithm (**Figure 1**). A direct significant correlation was observed between VISUAL and severity score (p=0.007). The increase in 1 unit (+) in VISUAL induced a positive correlation of severity score in 1.33-fold increase (95% CI: 1.08;1.57, p=0.007).

The ROC analysis indicated a good performance of VISUAL for the discrimination between Cluster A and B of severity score. When we tested VISUAL performance in predicting severe complications (Cluster B) compared to that of the isolated determination of smB lymphocytes [4,27,36], we found that VISUAL showed significantly higher sensitivity (85% *vs* 55%, p=0.01) and negative predictive value (77% *vs* 58%, respectively), while similar positive predictive value between both tests (72% *vs* 71%, respectively) and specificity (60% vs 73%, respectively) between both tests. The AUC obtained using the VISUAL score was superior to that derived using the isolated determination of smB lymphocytes, without statistical significance (AUC 0.721 *versus* 0.641) (**Figure 2**).

Severity Cluster A (<14 points) showed markedly lower VISUAL values (median 9 points, IQR 4). This could be pointing to a milder form of CVID or to a less evolved disease. Severity Cluster B (≥14 points) showed high VISUAL values (median 11 points, IQR 3). We evaluated time of evolution of the disease for each patient and did not observe statistically significant differences between clusters (data not shown).

Patients at diagnosis of CVID with VISUAL ≥10 (p30 of severity score) associated 8.94-fold higher odds of severe clinical prognosis (Cluster B) with respect to VISUAL below this threshold (p=0.001). In this study, Bootstrap 95% confidence intervals for the VISUAL score were as follows: sensitivity 85% (68-100%), specificity 60% (33-74%), and AUC of 0.721 (0.545 to 0.846).

Time to progress to cluster B of severity was assessed by Kaplan-Meier estimates for the VISUAL score. CVID patients with VISUAL≥10 points progressed to cluster B faster than those with VISUAL <10 (P = .0002) **(Figure 3),** with a median age of 37-years-old

(*versus* 44 years-old in patients with VISUAL<10, n=4). The mean time from age at CVID diagnosis to age at cluster B-progression was 2 years (median 0, IQR 1).

DISCUSSION

The novel VISUAL score, using combined immunological biomarkers at CVID diagnosis, early predicted the severity of clinical manifestations or outcomes in our CVID cohort, being independent of the course of the disease, with sensitivity of 85% and negative predictive value 77%. VISUAL showed superior sensitivity and accuracy to predict severity than the surrogate marker routinely used in clinical practice, namely smB phenotype alone. A cut-off of VISUAL 10 properly discriminated CVID patients with severe outcome (Cluster B). In our series, a large proportion of CVID patients showed severe manifestations (27/50, 54%), maybe translating infradiagnosis of mild cases in our health areas.

The VISUAL score is a non-invasive and easy-to-perform screening tool that would allow the identification of CVID patients at risk of presenting severe complications and that would benefit from close clinical follow-up and therapy. These results support the hypothesis that the clinical manifestations and therefore the increased risk of complications in CVID patients is associated with deeper alteration of the analytical biomarkers early at diagnosis. smB lymphocytes are a predictor of inflammatory complications and outcomes in CVID, while do not predict infectious complications [4,28]. More common infectious complications in CVID seem to be better predicted by functional tests of specific Ab production and by serum IgA [28,37], while $CD4^+$ T-lymphocytopenia may add on susceptibility risk in a subset of CVID patients. Several studies have considered specific antibodies' production within the assessment and especially in the IgRT decision for CVID patients [10], which identify infectious risk mainly to encapsulated bacteria and respiratory viruses. Besides, $CD4^+$ T lymphocyte stages can guide clinicians on problematic complications, such as opportunistic and recurrent viral infections, gastrointestinal disease, lymphoma, autoimmunity and inflammation in CVID patients [29,38,39]. Interestingly, up to 30% of our patients had severe infectious complications impacting their prognosis, which would have not been covered by smB lymphocytes phenotyping alone. This aspect highlights the necessity of combining markers to better define prognostic risk in CVID and the opportunity to avoid unfavorable evolution by using diagnostic and therapeutic strategies through early intervention. Thus, all 4 immunological variables scores integrate important information to CVID categorization. One of the weaknesses of this study is the relatively small size

of the cohort. However, due to the results obtained, it would be of interest to validate the VISUAL in a broader cohort of patients.

The application of scores represents a better comprehensive assessment of the clinical expression of a disease compared to isolated data. Likewise, the global staging generated by these scores allows a more homogeneous analysis of the results and enable to compare, predict and thus anticipate medical approaches in favor of individual patients. The proposed extended prognostic score proved to be a useful tool to classify CVID patients at diagnosis in order to anticipate and adjust follow-up and management. Notwithstanding that it is complicated to establish a linear distribution in all patients from a real-life scenario, so the performance of any instrument should be evaluated in individuals with quiescence or mild disease activity separately from patients with moderate to severe disease activity. A VISUAL of 10 points or above suggests the need to establish a closer monitoring, as well as to request specific studies according to the phenotype of each patient, especially those in Cluster B. Time to progression to cluster B of severity in CVID patients with VISUAL≥10 points progressed to cluster B faster than those with VISUAL <10 (P =.0002). A major question is whether VISUAL might change over time, due to changes in $CD4^+$ T lymphocytes numbers.

In conclusion, we performed a two-step clustering process of CVID patients to define prognosis by clinical-immunological correlation. Stratification of CVID patients by a combination of clinical and analytical scores is intended to cover the many different complications of the disease. VISUAL prognostic score might allow to predict and thus better establish the follow-up requirement of each individual patient, and would allow to modify the medical actions and control in a personalized way if necessary. Since the main purpose of scores is to predict severity, this score might contribute to earlier and closer management and follow-up of high-risk patients. We believe that this scoring tool may complement existing approaches and might impact clinical decisions and to provide a more personalized patient care.

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AUTHORSHIP CONTRIBUTIONS

K.G.-H. and S.S.-R. designed the study, analyzed the data and wrote the manuscript. A.J-H contributed to the design of the methodology and statistical analysis of data and figures. J.V and E.N contributed to the writing of the manuscript. All authors reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

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TABLES.

Table 1. VISUAL score of combined analytical biomarkers at diagnosis.

POINT VALUE	**1**	**2**	**3**	**4**
smB lymphocytes (%)	Normal	< 8%	< 2%	< 1%
IgA (g/L)	Normal-2SD	< 2SD	-	< 0.07
Specific Ab responses	Normal	Altered only polysaccharide or only protein responses		Altered polysaccharide and protein responses
$CD4^+$ T-lymphocytes (µ/mL)	700-1,500	500-700	200-500	<200

Suppl. Table 1. Clinical manifestations of the patients who presented a severity score greater than 14 according to Ameratunga et al.[11].

Patiens with Severe Score ≥ 14	Clinical Manifestation
1	Cytopenia requiring treatment, mild bronchiectasis, uncomplicated shingles, asymptomatic splenomegaly, acute sinusitis.
2	Food and antibiotic allergies, mild bronchiectasis, chronic rhinosinusitis, asymptomatic splenomegaly, Helicobacter pylori responding to treatment.
3	Cytopenias requiring treatment, multiple allegies antibiotic and reactions to IVIG uncomplicated pneumonia.
4	Mild bronchiectasis, non-life threatening abscesses, mild eczema, uncomplicated shingles.
5	Mild bronchiectasis, complicated pneumonia, asymptomatic splenomegaly, chronic rhinosinusitis.
6	Cytopenias requiring treatment, symptomatic splenomegaly, Helicobacter pylori responding to treatment, uncomplicated vitamin deficiency, uncomplicated CMV viremia, otitis externa.
7	Malignancy (recurrent non Hodgkin lymphoma plus Hodgkin lymphoma)
8	Severe GLILD, viral hepatitis responding to treatment, symptomatic splenomegaly, mild asymptomatic citopenias
9	Addison´s disease, multiple antibiotic allergies, osteomyelitis, asymptomatic splenomegaly.
10	Mild inflammatory bowel disease, Addison´s disease, otitis media, uncomplicated pneumonia, asymptomatic splenomegaly, mild asymptomatic cytopenias.
11	Reaction to IVIG, cytopenias requiring treatment, asimptomatic splenomegaly otitis media, uncomplicated UTI´s, uncomplicated neumonia.
12	Complicated pneumonia, sinusitis, mild bronchiectasis, Helicobacter pylori responding to treatment, symptomatic increase in liver enzimes, otitis externa.
13	Severe GLILD (pulmonary), viral hepatitis responding treatment, cutaneous vasculitis.

14	Malignancy (non Hodgkin lymphoma), uveitis responding to treatment, uncomplicated neumonia, arthritis.
15	Myositis, mild bronchiectasis, symptomatic splenectomy, acute sinusitis, mild eczema.
16	Primary biliary cirrhosis with liver transplantation, cytopenias requiring treatment, asymptomatic splenomegaly, uncomplicated neumonia.
17	Malignancy (gastric cancer), cytopenias requiring treatment, uncomplicated neumonia.
18	Chronic renal failure, type 1 diabetes, uncomplicated neumonia.
19	Malignancy (non Hodgkin lymphoma), connective tissue disorder, uncomplicated neumonia.
20	Severe SLE, cytopenias requiring treatment, giardia, uncomplicated UTI´s.
21	Non-life threatening abcesses, autoimmune gastritis, complicated pneumonia, autoimmune thyroiditis.
22	Complicated mastoiditis, complicated neumonia
23	Complicated neumonia, mild bronchiectasis, asymptomatica splenomegaly, mild asyptomatic citopenias, Giardia, acute sinusitis
24	Severe pulmonary dysfunction, complicated neumonia
25	Severe malabsorption protein-losing enteropathy, cytopenias requiring treatment, Giardia.
26	Complicated neumonias, mild bronchiectasis, chronic rhinosinusitis.
27	Meningitis, symptomatic splenomegaly, oral ulceration, mild limphadenopathy, mild asthma, acute sinusitis.

FIGURE LEGENDS.

Figure 1. Silhouette plots for the analysis of the two different clusters against VISUAL. Created with BioRender.com

Figure 2. Receiver operating characteristic (ROC) curves for VISUAL versus isolated smB lymphocytes determination as a predictor of severity manifestations in CVID patients. Abbreviation: AUC, area under the curve.

Figure 3. Time to progress to cluster B of severity was assessed by Kaplan-Meier estimates for the VISUAL score. CVID patients with VISUAL≥10 points progressed to cluster B faster than those with VISUAL <10 (P = .0002).

Suppl. Fig. 1. Density plots of Ameratunga´s severity score *versus* VISUAL score.

Figure 1.

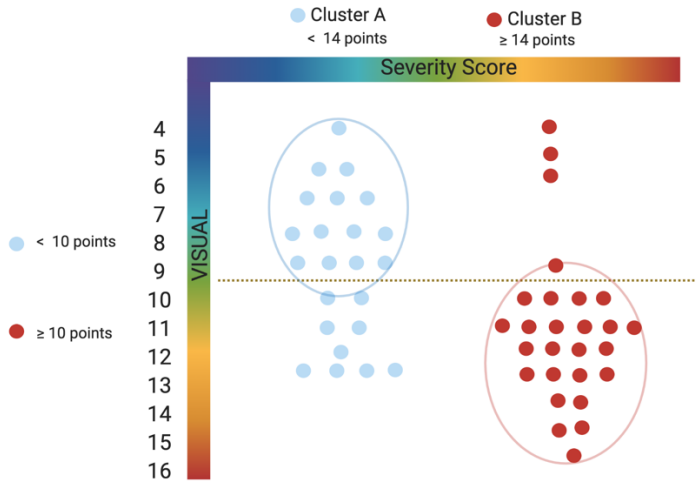

Cluster A
< 14 points
Cluster B
≥ 14 points
Severity Score
VISUAL
4
5
6
7
8
9
10
11
12
13
14
15
16
< 10 points
≥ 10 points

Figure 2

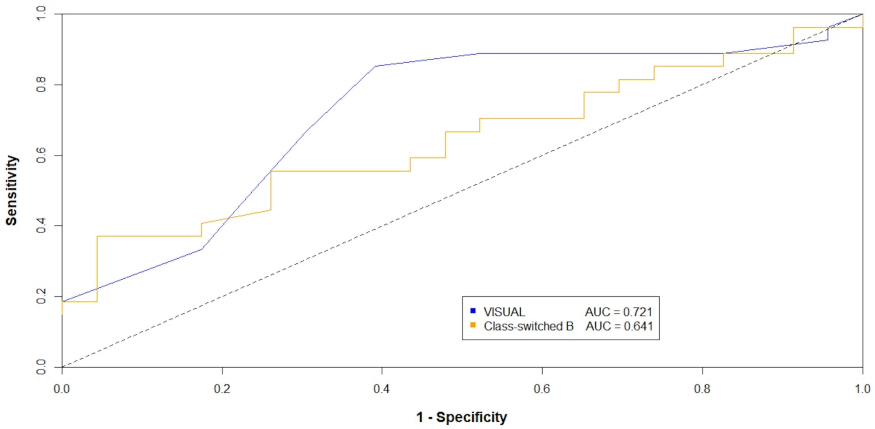
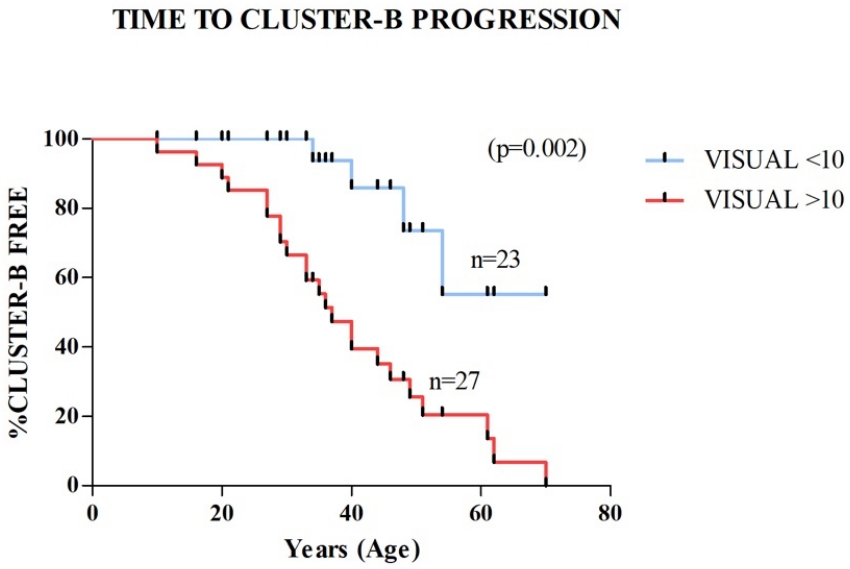
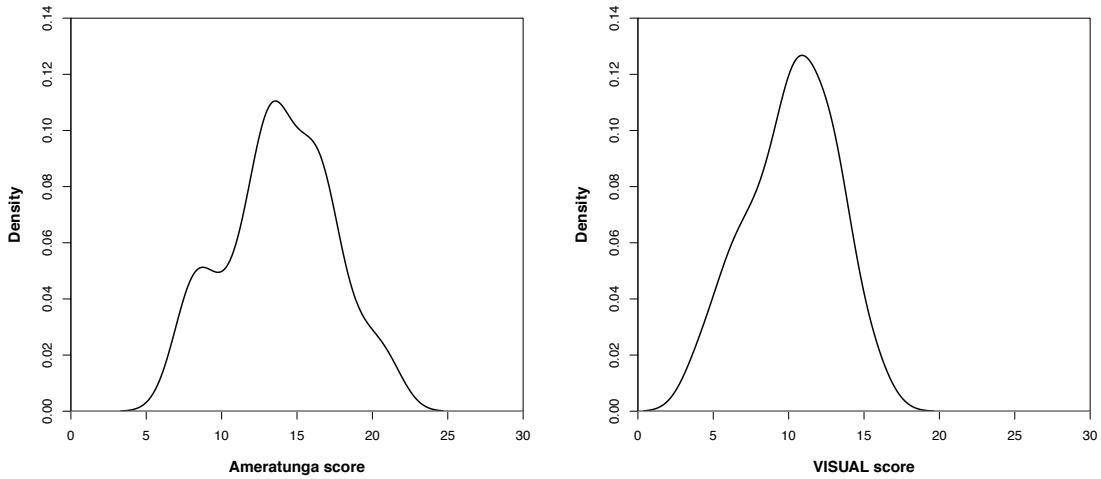

Sensitivity
1 - Specificity
0.0
0.2
0.4
0.6
0.8
1.0
VISUAL AUC = 0.721
Class-switched B AUC = 0.641

Figure 3.


TIME TO CLUSTER-B PROGRESSION
%CLUSTER-B FREE
100
80
60
40
20
0
0
20
40
60
80
Years (Age)
(p=0.002)
n=23
n=27
VISUAL <10
VISUAL >10

Suppl. Fig. 1.


Density
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0
5
10
15
20
25
30
Ameratunga score
Density
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0
5
10
15
20
25
30
VISUAL score