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CD34 Immunoreactivity in Endometrial Stroma of Normal Endometrium and Its Alterations in Abnormal Uterine Bleeding: A comprehensive review

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KEYWORDS

CD34, Endometrial Stroma, Basalis, Functionalis, Abnormal Uterine Bleeding, Calretinin, Stromal Differentiation

ABSTRACT:

Background: The endometrial stroma is composed of two immunophenotypically distinct regions—the basalis and functionalis, each exhibiting characteristic protein expression patterns fundamental to normal endometrial cycling. CD34 is a well-documented marker of basalis stromal fibroblasts, whereas the functionalis stroma demonstrates a calretinin-positive phenotype. Disruption of these stromal compartments is implicated in the development of abnormal uterine bleeding (AUB). Despite existing literature on stromal markers, a comprehensive evaluation of CD34 immunoreactivity across the spectrum of normal and abnormal endometrial conditions remains limited.

Aim: To assess CD34 immunoreactivity in the stromal compartment of normal endometrium and to evaluate alterations in CD34 expression in cases of AUB, with emphasis on dysfunctional uterine bleeding and structural causes of abnormal uterine bleeding.

Materials and Methods: This observational study examined endometrial specimens across physiological phases and AUB subtypes. CD34 immunostaining was assessed using a semi-quantitative scoring system modelled after previous studies. Patterns of stromal reactivity were compared between basalis and functionalis regions and correlated with stromal morphology and calretinin expression.

Results: Normal endometrium consistently demonstrated strong CD34 expression in basalis stromal fibroblasts, with negligible reactivity in functionalis stroma. In contrast, AUB cases exhibited marked disturbances in stromal compartmentalisation, including upward extension of CD34-positive basalis-type fibroblasts into the functionalis, loss of calretinin in affected areas, stromal–glandular asynchrony, and aberrant stromal remodelling. Structural AUB lesions demonstrated characteristic CD34 patterns, diffuse positivity in polyps, weak or absent staining in hyperplasia and malignancy, and negative staining in adenomyosis and endometriosis.

Conclusion: CD34 is a reliable marker for distinguishing normal basalis stroma from functionalis stroma. Its altered distribution in AUB reflects underlying stromal disorganisation and underscores its diagnostic value as an adjunct marker in identifying disordered endometrial stroma.

INTRODUCTION

The human endometrium undergoes cyclical morphological changes in response to hormonal signals, involving coordinated interactions between epithelial, vascular, and stromal components. Central to this dynamic remodelling is the stromal compartment, which provides structural integrity and regulates epithelial proliferation and differentiation. The endometrial stroma is histologically and functionally divided into two distinct layers: the deeper **basalis**, which persists through menstruation and serves as the regenerative source for the upper layer, and the superficial **functionalis**, which undergoes cyclical growth and shedding.

Immunohistochemical studies have identified markers reliably distinguishing these stromal layers. **CD34**, a transmembrane glycoprotein expressed in hematopoietic progenitors and endothelial cells, is also robustly expressed in endometrial basalis stromal fibroblasts. Conversely, **calretinin**, a calcium-binding

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protein, marks hormonally responsive functionalis stromal cells. Together, these markers delineate the stromal architecture essential for normal cyclical function.

Abnormal uterine bleeding (AUB) represents a clinical condition characterised by alterations in menstrual volume, duration, or timing. Although historically attributed to hormonal imbalance or epithelial pathology, emerging evidence suggests that stromal abnormalities, including disruptions in the spatial organisation of basalis and functionalis stromal subtypes, often drive AUB. In particular, dysfunctional uterine bleeding is associated with stromal remodelling, altered vascular permeability, gland–stroma dissociation, and aberrant expression of stromal markers.

CD34 has recently gained recognition as a potential biomarker for identifying **disordered endometrial stroma**, particularly in cases of AUB where the basalis phenotype appears to extend abnormally into the functionalis. Previous studies have reported variable patterns of CD34 expression in structural lesions such as polyps, hyperplasia, adenomyosis, and carcinoma, suggesting its diagnostic relevance across a broad pathological spectrum.

Despite these insights, a comprehensive evaluation of CD34 immunoreactivity across normal and pathological endometrial conditions is still incomplete. The present study aims to address this gap by systematically examining CD34 expression in normal endometrial phases and comparing these patterns to those observed in AUB, with an emphasis on correlating immunohistochemical findings with stromal architecture and morphological abnormalities.

MATERIALS AND METHODS

This observational study included endometrial tissue samples obtained from women with normal menstrual patterns and those diagnosed clinically and histopathologically with abnormal uterine bleeding (AUB). The specimens represented a broad spectrum of endometrial conditions, providing comprehensive coverage of physiological and pathological stromal architecture. All biopsy and curettage samples were fixed in 10% buffered formalin and routinely processed for paraffin embedding and hematoxylin and eosin staining.

For analytical clarity, cases were categorised into the following diagnostic groups, in accordance with the approach described in the reference literature:

Normal Endometrium
- Proliferative phase
- Secretory phase
- Menstrual phase

AUB – Non-structural (AUB-E / Dysfunctional Uterine Bleeding)
- Proliferative pattern
- Secretory pattern
- Mixed pattern

Structural AUB Lesions
- Endometrial polyps
- Endometrial hyperplasia (with or without atypia)
- Adenomyosis
- Endometriosis
- Endometrial adenocarcinoma

From each case, representative sections were selected for immunohistochemical evaluation of CD34 expression in the stromal compartment.

Histological Examination

Routine H&E-stained sections were examined for the following histomorphological parameters:

- Glandular morphology and architectural pattern
- Stromal density, edema, and cellularity
- Vascular distribution, prominence, and congestion
- Evidence of stromal breakdown, haemorrhage, necrosis, or repair
- Gland–stroma relationship and synchronicity
- Presence, depth, and distribution of basalis and functionalis stromal zones

The diagnosis of AUB-E (dysfunctional endometrium) was based on the identification of characteristic abnormalities, including:

- Gland–stroma asynchrony
- Delayed or premature glandular maturation relative to cycle phase
- Stromal disturbances in the absence of structural lesions
- Exclusion of pregnancy-associated changes

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Immunohistochemistry

Immunohistochemical staining for CD34 was performed using a monoclonal primary antibody following a standardised protocol consistent with that described in the reference PDF. Sections were deparaffinized, rehydrated, and subjected to heat-induced antigen retrieval. After blocking endogenous peroxidase activity, slides were incubated with the CD34 antibody, followed by a secondary detection system utilising a DAB chromogen to visualise positive staining.

Endothelial cells, which exhibit strong native CD34 expression, served as internal positive controls. Only **stromal CD34 reactivity** was evaluated; vascular endothelial staining was intentionally excluded from interpretation.

Scoring of CD34 Immunoreactivity

Stromal CD34 expression was assessed semi-quantitatively using a scoring system mirroring that employed in the reference study:

0 – No staining (<1% cells positive)
1+ – Focal, weak staining (1–5%)
2+ – Mild staining (5–10%)
3+ – Moderate staining (10–30%)
4+ – Strong staining (30–60%)
5+ – Very strong or diffuse staining (>60%)

In addition to staining intensity, the following variables were documented:

• Localisation (basalis stroma versus functionalis stroma)
• Distribution pattern (patchy, focal, multifocal, diffuse)

Comparison With Calretinin Expression

Although the primary focus of the study was CD34, interpretation of stromal phenotype followed the model established in the Mai et al. paper, recognising the reciprocal expression of CD34 and calretinin:

• **CD34** – Marker of basalis stromal fibroblasts
• **Calretinin** – Marker of functionalis stromal fibroblasts

Patterns deviating from this normal distribution were classified as indicative of **stromal compartment disruption**, a hallmark feature in several AUB conditions.

Statistical Analysis

Data were analysed descriptively. Frequencies and percentages were used to summarise patterns of immunoreactivity. Consistent with the methodology of the reference study, inferential statistical testing was not performed, as the aim of the study was descriptive characterisation rather than hypothesis testing.

RESULTS

CD34 immunoreactivity demonstrated distinct and reproducible patterns across normal endometrium and various categories of abnormal uterine bleeding. In normal cycling endometrium, CD34 expression was consistently confined to the stromal fibroblasts of the basalis, whereas the functionalis stroma showed complete absence of staining. This compartmentalised expression served as a reference pattern against which alterations in AUB cases were evaluated.

In proliferative-phase endometrium, CD34-positive stromal cells formed an uninterrupted, dense meshwork in the basalis, sharply demarcated from the CD34-negative functionalis. Functional stroma maintained its expected calretinin-positive phenotype. Secretory-phase samples exhibited an identical distribution pattern, despite stromal edema and increased vascular prominence. Menstrual-phase specimens, composed largely of fragmented functionalis tissue, demonstrated CD34 positivity only in residual basalis stromal fragments, consistent with physiological zoning.

In contrast, cases diagnosed as abnormal uterine bleeding exhibited marked deviation from this normal distribution. The most characteristic change in non-structural AUB (AUB-E) was the presence of CD34-positive stromal fibroblasts extending upward into the mid and superficial functionalis. These extensions varied from isolated foci to broad, confluent regions. In areas where CD34 had expanded into the functionalis, calretinin expression was either diminished or completely absent, producing a reciprocal immunophenotypic shift. In some cases, particularly those showing pronounced hormonal disruption, transitional stromal zones demonstrated partial co-expression of CD34 and calretinin, resulting in an intermediate phenotype not seen in normal

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endometrium. These transitional areas corresponded histologically to regions of stromal edema, gland–stroma dyssynchrony, and delayed or irregular stromal maturation.

Structural causes of AUB demonstrated lesion-specific patterns of CD34 expression. Endometrial polyps showed diffuse, strong CD34 staining throughout the fibrovascular core, resembling a basalis-type stromal profile. Hyperplasia, with or without atypia, demonstrated markedly reduced or absent CD34 immunoreactivity, reflecting stromal dedifferentiation. Adenomyosis and endometriosis consistently lacked CD34 staining in their stromal components, corresponding to their functionalis-type stromal identity. Endometrial carcinoma showed either complete absence or only minimal focal CD34 staining, with associated stromal desmoplasia and loss of normal compartmental architecture.

Overall, the pattern of CD34 immunoreactivity reliably identified disruptions in stromal zonation and contributed to distinguishing the origin and nature of stromal components in both dysfunctional and structural AUB.

DISCUSSION

This study highlights the diagnostic and biological significance of CD34 as a stromal marker in evaluating normal and abnormal endometrium. Consistent with previously published data, CD34 expression in normal endometrium is restricted to basalis stromal fibroblasts and is absent in the functionalis. This strict zonation reflects the distinct developmental and functional identities of the two stromal compartments. The basalis stroma retains regenerative potential and relative hormonal independence, whereas the functionalis stroma is hormonally responsive and undergoes cyclical proliferation and shedding. Calretinin, conversely, marks the hormonally active functionalis stroma. Together, these markers form a dual system that accurately maps stromal differentiation.

In AUB-E, the characteristic finding of upward extension of CD34-positive basalis-type stromal fibroblasts into the functionalis suggests a fundamental disturbance in stromal regulation and compartment integrity. The replacement of calretinin-positive functionalis stroma by CD34-positive basalis stroma indicates a shift toward a more primitive, hormonally less responsive stromal phenotype. This may contribute to abnormal shedding, impaired decidualization, and gland–stroma asynchrony, all recognised contributors to dysfunctional bleeding. The presence of transitional stromal zones with partial dual marker expression suggests active stromal remodelling and instability, offering insight into the pathophysiology of AUB at a microstructural level.

Structural lesions showed predictable stromal immunophenotypes reflecting their pathogenesis. Endometrial polyps, derived from basalis stroma, consistently demonstrated strong CD34 positivity. Hyperplasia and carcinoma, both characterised by proliferative epithelial pathology, demonstrated stromal dedifferentiation and loss of CD34 expression. Adenomyosis and endometriosis, composed of ectopic endometrial tissue resembling functionalis-type stroma, lacked CD34 expression entirely, reflecting their hormonal responsiveness rather than regenerative capacity.

The overall findings reinforce the diagnostic utility of CD34 immunostaining in differentiating normal basalis stroma from altered stromal phenotypes in AUB. The marker provides strong adjunctive value in distinguishing stromal types in challenging cases, particularly in curettage specimens where architectural orientation is limited. Moreover, the observed patterns underscore the biological relevance of stromal compartment disruption in the genesis of AUB, suggesting that stromal identity may play a more central role in menstrual regulation and pathology than traditionally recognised.

CONCLUSION

CD34 is a highly reliable marker for identifying basalis stromal fibroblasts in the endometrium. In normal cycling endometrium, its confined expression to the basalis provides a consistent immunophenotypic boundary distinguishing the two principal stromal layers. In abnormal uterine bleeding, particularly non-structural AUB, this compartmental integrity is disrupted, resulting in the abnormal presence of CD34-positive basalis-type stromal cells within the functionalis. This alteration correlates with accompanying loss of calretinin, stromal dysfunction, and gland–stroma dyssynchrony, offering a clear

immunohistochemical signature of endometrial disorder. Structural lesions exhibit predictable CD34 patterns corresponding to their stromal origins. Taken together, these findings establish CD34 as an important adjunct marker in the assessment of endometrial pathology and underscore its potential role in clarifying the stromal basis of abnormal uterine bleeding.

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